CANADIAN JOURNAL OF RESEARCH

VOLUME 14 NOVEMBER, 1936 NUMBER 11

CONTENTS

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

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The Canadian Journal of Research is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. All correspondence should be addressed:

National Research Council, Ottawa, Canada

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 14, SEC. C. NOVEMBER, 1936 NUMBER 11

STATISTICAL SIGNIFICANCE OF WHEAT PROTEIN PERCENTAGE DIFFERENCES IN VARIETAL TRIALS[1]

BY A. G. O. WHITESIDE[2]

Abstract

The results of protein determinations on 28 varieties of spring wheat grown in quadruplicate rod-row plots at each of three Dominion Experimental Stations were analyzed statistically. The error due to plot variability greatly exceeded the laboratory error. No real differences were found between the calculated percentages for composite samples made up from the four plots of each station and the percentages obtained by averaging the results from the individual plots.

Some of the varieties showed definite tendencies towards high protein content. The major environmental effects of station and of replication gave negative correlations between yield and protein content but when these major factors together with the influence of variety were removed, yield and protein content were not correlated.

Introduction

In the relative classification of wheat varieties on the basis of wheat protein percentage, a knowledge of the accuracy of the test is important. Numerous investigators have pointed out that the quantity of protein found in wheat is greatly influenced by environment, and those familiar with appraising the relative quality of wheat varieties recognize that good field plot technique is as important for quality comparisons as it is for yield comparisons.

Newton and Malloch (1) demonstrated that wide variations may occur in the protein content of wheat grown in replicate plots and that single plots were unreliable for making varietal comparisons. In a series of 12 varieties grown at Raymond, Alberta, in 1/200 acre plots with four plots allotted to each variety, an average spread of 3.2% was found between the high and the low values for the plots of each variety. In another series of plots grown at Edmonton the average spread for all varieties was 1.1%. They conclude that "whatever method of replication or sampling is used, however, it is unsafe to attach importance to small differences unless these can be proven statistically to be significant."

In recent years plant breeders in Canada have more or less adopted the rod row method of variety testing first used in the United States. The plots are small in size and where close comparisons are desired the varieties in a group are few in number in order not to spread the experiment over too great an area of land. Varietal competition and border effect are taken care of by

[1] *Manuscript received September 15, 1936.*
Contribution from the Cereal Division, Experimental Farms Branch, Ottawa. Issued as Paper No. 102 of the Associate Committee on Grain Research of the National Research Council of Canada and the Dominion Department of Agriculture.

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including only the inner rows of a plot and excluding the end plants of each row at harvest. Usually four replications are used for each variety and the manner of plot arrangement is such that Fisher's (2) analysis of variance may be applied to the data.

In 1935 a series of 28 varieties of spring wheat was grown in replicated rod row plots at each of three Dominion Experimental Stations. These are located at Swift Current and Scott, Saskatchewan, and at Lacombe, Alberta. Each plot consisted of 5 rows, 7 inches apart and $18\frac{1}{2}$ ft. long. The plots of the 28 varieties were randomized within a replication and 4 replications were grown at each station. The grain harvested from each plot was taken from the 3 inner rows which measured $16\frac{1}{2}$ ft. in length. The protein determinations were made by the Chemistry Division.

Laboratory Error

Fifty-gram samples were finely ground in a Hobart grinder; the protein percentages were determined in duplicate on each sample and the results calculated to 13.5% moisture basis. There were 112 samples from each station making 336 samples in all.

The statistical analysis of the data (Table I) indicated that protein percentages may be determined in the laboratory within narrow limits of variation. A difference of approximately 0.3 in percentage protein is necessary in order to be statistically significant in this series. It might be pointed out that the difference in the laboratory error for the three series is not significant, thus justifying the calculation of a single standard error for the entire series of determinations.

TABLE I

LABORATORY ERROR FOR WHEAT PROTEIN PERCENTAGES

	Swift Current	Scott	Lacombe	All Stations
S.E. (single determination)	.1503	.1559	.1387	.1485
S.E. (for mean of a variety)	.1063	.1102	.0982	.1050
N.D.* ($2 \times \sqrt{2} \times$ S.E. m)	.3007	.3117	.2777	.2969

* *Necessary difference for significance.*

Sampling or Plot Error

If the laboratory error were used as a basis for comparing varieties in their ability to produce high protein percentages it would at once be open to criticism, since there is no account taken of the variability occurring in the wheat from different plots. The analysis of variance was, therefore, calculated and the data secured are presented in Table II. The calculated necessary difference between the means of any two varieties at each of the three stations varied from 0.41 to 0.74 in protein percentage. The variances for varieties and for replications were removed from the total variance in calculating the necessary differences. The total variability, it will be noted, has been sub-

divided into three parts, namely, the varietal effect, the environmental effect attributed to replications, and that which remains, or not accounted for by either varieties or replications. It will also be observed from the F values that the variances for varieties and for replications at each of the stations were significant, demonstrating that real differences existed due to varietal effects as well as to environmental effects.

TABLE II

SAMPLING ERROR FOR DIFFERENCES BETWEEN VARIETIES AT EACH STATION

Variation due to	S.S.	D.F.	Variance	F	*5% Pt.	*1% Pt.
Swift Current						
Varieties	43.08	27	1.595	5.89	1.65	2.03
Replications	3.46	3	1.155	4.27	2.72	4.04
Remainder	21.92	81	.2706			
Scott						
Varieties	30.87	27	1.144	13.90	1.65	2.03
Replications	1.10	3	.366	4.45	2.72	4.04
Remainder	6.67	81	.0823			
Lacombe						
Varieties	75.35	27	2.791	27.84	1.65	2.03
Replications	1.32	3	.439	4.38	2.72	4.04
Remainder	8.13	81	.1003			

	Swift Current	Scott	Lacombe
Mean protein in %	15.31	15.97	13.96
S.E. (single determination)	.5202	.2869	.3167
S.E. (for mean of a variety)	.2601	.1435	.1583
N.D. ($2 \times \sqrt{2} \times$ S.E. m)	.7357	.4059	.4477

* *Approximate. Taken from Snedecor's tables (3).*

Application of the calculated necessary difference to the wheat protein percentages for each variety at each station (see Table IV) will indicate the varieties which are significantly higher than others in this characteristic at each station. To study the behavior of the varieties for the three stations combined, the calculations were extended to include all the data. In Table III it will be noted that significant values were obtained for varieties, replications, stations and for interaction of varieties and stations. The significant value for the interaction of varieties and stations indicates that the varieties did not behave exactly the same in relation to each other at all three stations. Since the necessary difference calculated from the interaction variance for varieties and stations is larger than the necessary difference calculated from the error variance for the three stations, then the larger error should be used to predict whether the differences observed are consistent for the three localities and are likely to be of the same order if the test were repeated.

TABLE III

SAMPLING ERROR FOR DIFFERENCES BETWEEN VARIETIES FOR THE THREE STATIONS

Variation due to	S.S.	D.F.	Variance	F	5% Pt.	1% Pt.
Varieties	115.92	27	4.29	29.2	1.56	1.87
Replications	5.88	9	.6532	4.4	1.96	2.56
Stations	236.52	2	118.26	804.7	3.04	4.70
Varieties × Stations	33.39	54	.6183	4.2	1.97	2.60
Remainder	35.71	243	.1470			

Necessary differences based on error variance	
S.E. for single determination	.3834
S.E. for mean of a variety	.1107
N.D. ($2 \times \sqrt{2} \times$ S.E. m)	.3131

Necessary differences based on variance for interactions of varieties and stations	
S.E. for single determination (4 plots)	.3934
S.E. for mean of a variety	.2270
N.D. ($2 \times \sqrt{2} \times$ S.E. m)	.6420

TABLE IV

MEAN PROTEIN PERCENTAGES FOR THE 28 VARIETIES TOGETHER WITH A CLASSIFICATION BASED ON NECESSARY DIFFERENCES

Variety	Mean for 3 Sta.		Swift Current		Scott		Lacombe	
	Protein, %	Class*	Protein, %	Class**	Protein, %	Class**	Protein, %	Class**
Reward 26–32	15.97	1	16.23	1	16.58	2	15.10	3
Reward Ott. 928	15.75	1	15.63	0	16.45	1	15.18	3
Reward 26–43	15.74	1	15.70	0	16.65	2	14.88	3
Reward Morden	15.74	1	15.90	1	16.28	1	15.05	3
Reward 28-25-1	15.73	1	15.80	0	16.20	1	14.95	3
944-A-33-11	15.69	1	16.05	1	16.75	3	14.25	0
Reward 22–42	15.67	1	15.95	1	16.28	1	14.78	2
Reward 22–35	15.62	1	15.65	0	16.28	1	14.93	3
Reward Long	15.61	1	15.83	1	16.18	0	14.83	2
Reward 3-25-A	15.56	1	15.65	0	16.30	1	14.70	2
Reward M × R R	15.55	1	15.70	0	16.08	0	14.88	3
944 A	15.46	0	16.05	1	16.50	1	13.83	0
Thatcher	15.14	0	15.75	0	16.28	1	13.40	−1
S.C. 26-264	15.10	0	15.13	0	15.90	0	14.28	0
G × P 2-27	14.96	0	15.45	0	16.08	0	13.35	−1
1325-29	14.91	0	14.78	−1	16.40	1	13.55	−1
Marquis	14.91	0	15.15	0	15.50	−1	14.08	0
M × P 1-27	14.82	0	15.03	0	15.75	0	13.68	−1
S.C. 26-268	14.81	0	15.05	0	15.50	−1	13.83	0
M × P 3-27	14.81	0	15.15	0	16.28	1	12.90	−3
M × P 7-27	14.76	0	15.20	0	16.00	0	13.08	−2
1319-6	14.69	0	15.13	0	15.68	0	13.28	−2
P × G 1-27	14.65	−1	15.25	0	15.75	0	12.95	−3
Garnet	14.31	−1	14.90	0	15.43	−1	12.60	−4
1320-23	14.29	−1	14.50	−1	15.38	−2	13.05	−2
Red Bobs 222	14.18	−1	14.15	−2	15.23	−2	13.15	−2
Shanks	14.07	−2	14.20	−2	14.60	−4	13.40	−1
1320-18	13.92	−2	13.65	−3	14.93	−3	12.93	−3
Mean	15.08		15.31		15.97		13.96	
N.D.		.46*		.54**		.29**		.32**

* *Based on variance for interaction between varieties and stations.*
** *Based on variance for error at the same station.*

In Table IV the 28 varieties are classified in respect to protein percentage for each of the three stations and for the mean values for the three stations combined. Each variety was tested against the mean protein of the remaining 27 by calculating the necessary differences from the formula $2\sqrt{\frac{n+1}{n}}\ s^2$ where $n = 27$ and $s =$ the standard error for a mean of a variety. It is interesting to note that the 10 strains of Reward were not significantly different in the averages of the three stations and all were higher than Marquis. It might be mentioned here that the Reward variety has for many years consistently given higher protein percentages than Marquis.

Individual Samples *versus* Composite Samples

In the testing of new varieties of hard red spring wheat for different areas of Western Canada it is customary to conduct protein determinations on samples grown in plots at a number of stations. Composite samples from the replicated plots grown at each station are used for this purpose rather than the wheat from each plot, owing to the multiplicity of tests which would be involved. Unless some knowledge of the necessary differences expected at each station is available, it is rather difficult to estimate a reasonable error for prediction although it would appear from this experiment that it would not be large. Since agronomists do not place much reliance on the results from any one station for one year it would appear that the use of composite samples would be justified, especially if the composite sample had essentially the same protein content as the mean protein for the replicated plots of each variety. If variety tests are conducted at a number of locations in a given area it would be a simple matter to calculate the variance remaining after removing variety and location variance from the total. The necessary differences calculated in this manner would correspond to that derived from the variance for interaction between varieties and stations and should, therefore, give a reasonable basis for prediction. Variety tests conducted at a number of stations would likely give a higher error for interaction effects than the error of the experiment in a properly planned system of plots.

The manner in which composite samples should be made up for quality tests has often been a question. Should equal quantities of wheat from each plot be combined to make up a composite or should an aliquot be taken from the thoroughly mixed samples from all the wheat produced by the four plots at each station? In order to obtain sufficient wheat for a milling test the latter procedure is often the only feasible one owing to low yields which might occur in one or more plots. In this experiment an opportunity to examine the two methods of sampling in respect to the protein determination was afforded. The protein percentages for the four plots for each variety at each station were averaged and this was compared with the calculated average protein percentages based on the grams of protein produced per plot. In other words, the yield in grams per plot was multiplied by the protein percentage to obtain the amount of protein in grams. The total number of

grams of protein for the four plots was divided by the total yield, thus giving the percentage protein for the variety, which should correspond to what would be expected if the protein were determined on the composite sample from all the wheat produced by the four plots. The data revealed that the two sets of protein percentages were almost identical and gave a correlation coefficient of $r_{xy} = 0.9998$.

Yield *versus* Protein Percentages

To determine the relation between yield and protein percentages the variances and covariances were calculated for each station from which the correlations were obtained, as given in Table V. The total correlations were subdivided into the various components to measure the effect of each on the relation of yield to protein content. The total correlations for the Swift Current and Scott stations were not large and that for the Lacombe station was insignificant. When the effect of varieties was removed from the total correlations, insignificant correlations between yield and protein percentage were obtained for each of the stations. The correlations for replications were not significant although a negative tendency was indicated. This is not surprising since only two degrees of freedom were available for testing the significance of the correlations. The residual variations in yield per plot were therefore not associated with the residual variations in protein percentage and this would account in large part for the close relation between the mean protein percentages for the four plots of a variety and the calculated protein percentages based on the grams of protein produced per plot.

TABLE V

CORRELATIONS FOR YIELD (y) AND PROTEIN (p) CONTENT OBTAINED FROM THE VARIANCES AND COVARIANCES FOR EACH STATION

	D.F.	$y\ p$
Swift Current		
Total	110	−.2959*
Varieties	26	−.5816*
Replications	2	−.7478
Total—varieties	83	−.0002
Remainder	80	.1605
Scott		
Total	110	−.2077**
Varieties	26	−.3713
Replications	2	−.4445
Total—varieties	83	.0829
Remainder	80	−.1532
Lacombe		
Total	110	.1304
Varieties	26	.2393
Replications	2	−.8147
Total—varieties	83	−.1902
Remainder	80	−.0705

* *Greater than* $P = .01$.
** *Greater than* $P = .05$.

TABLE VI

CORRELATIONS FOR YIELD (y) AND PROTEIN (p) CONTENT OBTAINED FROM THE VARIANCES AND COVARIANCES FOR THE THREE STATIONS COMBINED

	D.F.	yp
Total	334	−.7046*
Varieties	26	−.0945
Varieties × Stations	53	.1774
Total—varieties	307	−.8249*
Stations	1	−.9991**
Replications	8	−.6420*
Remainder	242	.0390

* *Greater than* $P = .01$.
** *Greater than* $P = .05$.

In Table VI the correlations are presented for the three stations combined. The major effects of environment, as represented by the correlations for stations and replications, indicate that these are chiefly responsible for the significant total negative correlation. The correlations for varieties and interaction of varieties and stations contribute very little to the total correlation. When all of these are removed from the total correlation, yield and protein content are not significantly correlated.

The Quality Testing of Wheat Varieties

The quantity of protein is an important factor in quality of wheat and also serves as a guide in interpreting baking test results. While this experiment deals only with the variability occurring in protein percentage in varietal trials, it suggests a reasonable basis of sampling for tests designed to determine the suitability or unsuitability, in quality, of varieties for certain areas. Variability in protein content within varieties at each station does not appear to be large if a well planned system of plots is used. To obtain a cross section of the quality behavior of varieties for a given area without entailing too many samples for testing, equal quantities of wheat for each variety from each point might be composited, thus pooling the environmental effects for that area. The manner of compositing, however, would depend on the soundness or suitability of the samples for quality tests. It would, of course, be advantageous to make a complete analysis for each variety grown at each location for a fuller interpretation of the adaptability of the variety to the area. A uniform series of varieties tested in several areas in this manner should help in the zonation of varieties for different areas. This procedure is receiving attention in Canada at the present time, as numerous plot tests of the better wheat varieties are being conducted in farmers' fields in conjunction with the experimental stations.

Acknowledgments

The writer acknowledges the assistance of the various agencies referred to in this paper for contributing the samples and supplying the required data. The author is grateful to Dr. Goulden and others who have freely given advice in the handling of the data and to Mr. J. Edgar for assistance in making the calculations.

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PASTURE STUDIES XI.

PASTURE RESEARCH IN QUEBEC. CHEMICAL, ECOLOGICAL AND NUTRITIONAL PHASES[1]

By L. C. Raymond[2]

Abstract

Surveys of pasture flora in Quebec have led to the recognition of four layers, *viz.*, turf, tall herbaceous, shrub and tree. The human factor is advanced as the most important single agency determining the existing plant life but water supply and the fertility level exert important effects.

The soils under investigation carry sufficient seed to produce, on the average, 77 million potential plants per acre, but these seeds show a low correlation with the plants prevalent in the sward. It was revealed that each mature cow voids 1¼ million viable seeds per grazing season and that these seeds are closely related to the components of the sward.

Where phosphorus was applied as superphosphate at a rate of 700 lb. per acre, it was shown that most of that element was fixed in the top half-inch of soil and that the available portion of it was largely depleted after three crop years. Pot cultures of pasture soils growing either *Phleum pratense* or *Trifolium repens* show that calcium has depressed and sulphur has increased both the herbage yield and the uptake of phosphorus. The organic fraction of phosphorus in soils has been identified as containing 0.5% of lecithin and 65% of nucleic acid. The latter has been extracted quantitatively in pure form.

Mixed herbage and pure grass species have been fed to rabbits as a means of determining feeding value. The 35 groups fed have given highly variable results. Statistical examination of the data shows little if any correlation between gains and the constituents of the herbage as determined by a standard feeding-stuffs analysis. It has been tentatively concluded that the condition of the fibre, depending on the proportion of ligno and hemicellulose, is the most likely cause of the variability. The study is proceeding.

Introduction

Pasture research at Macdonald College had its inception in 1930. It very quickly became evident that there were involved a number of distinct phases which cut across ordinary departmental lines. As a result, a committee consisting of representatives of the departments of agronomy, animal nutrition, botany and chemistry was appointed in 1931, and has functioned since that time. Each member of the committee directs the work falling in his particular field.

The detailed features of the investigations are carried out by graduate assistants. At the present time three such men are appointed on a two-year basis, one in agronomy, one in animal nutrition and one in chemistry, with a part time botanical assistant. Each of the regular assistants gives full time in summer and half time during the winter months. This makes it possible for them to proceed to a higher degree in the two-year period. Some phase of the research work in progress is used as thesis material. The scheme has functioned very satisfactorily.

[1] *Manuscript received September 5, 1936.*
Contribution from the Macdonald College Pasture Committee, Macdonald College, Quebec. Presented at the Ottawa Meeting of the Royal Society of Canada, May, 1936.

[2] *Chairman, Macdonald College Pasture Committee, and Assistant Professor of Agronomy, Macdonald College.*

Much of the research undertaken has already been published. This paper is a résumé of the more recent findings and of some of the work in progress. The various aspects will be grouped as far as possible for presentation.

I. Soil Zonation

At the outset, a very tentative and general grouping of the soils of Quebec was available (13). The main divisions recognized, at that time, were the Laurentian upland podsols, the Appalachian upland podsols, the brown forest soils and the marine, lake and river group. All of these are still in need of much clarification. Until 1935 the major part of the field work was confined to the brown forest area and, as time permitted, the dividing line between this group and the podsols to the south and east was studied in some detail. Fig. 1 shows the result of the surveys made. The region covered is divisible into four main zones, (i) the upland Appalachian heavily leached, podsolized soils; (ii) the less heavily leached brown forest soils, 400 feet or more above sea level; (iii) an area with the soils transitional between the two above; and (iv) the lowland types. The U.S. soil types adjoining the international boundary are also indicated.

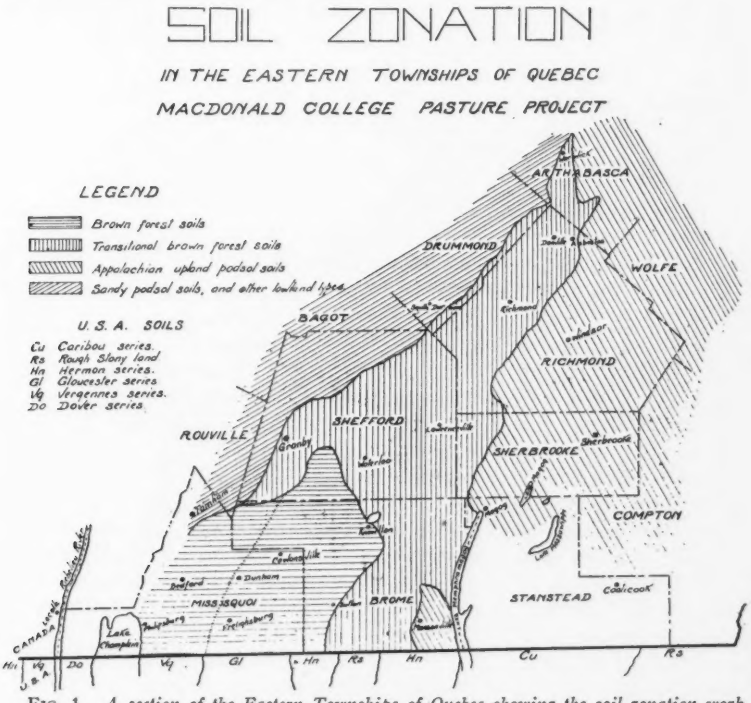


FIG. 1. *A section of the Eastern Townships of Quebec showing the soil zonation work accomplished to date.*

The nature of the two dominant soils in this locality has been quite fully discussed by McKibbin and Pugsley (12). The brown forest soils generally are deeper than the podsolized soils. In the former, organic and mineral matter are more intimately mingled, and the successive soil layers from the surface downward differ less markedly from one another than those of the podsols. In virgin podsols the surface few inches is very largely organic matter, with severely leached underlying material. Consequent upon their physical and chemical dissimilarity there are differences in biological response within and upon the soils of these zones.

II. Ecological Aspects

A. Survey

Following the soil classification the next logical step was to gain some knowledge of the existing flora. The first work of this nature was efforts by Newton and Nowosad (9) and Newton and Stobbe (10) to correlate vegetation with the various soil zones. They found, for example, that the brown forest soils were marked by deciduous woods composed chiefly of *Acer saccharum* mixed with *Fagus grandiflora*, *Betula lutea*, etc., and the podsols by mixed coniferous forest dominated in parts by *Picea canadensis*, *P. mariana*, and *Tsuga canadensis*, with *Acer saccharum*, *A. spicatum* and *A. pennsylvanicum* often present. They also listed some forest-floor plants and others of indicator value.

More recently, Whyte (16) has endeavored to make an ecological classification of the pastures, using as a basis of primary distinction the more conspicuous physiognomic features of the vegetation and working down to subdivisions requiring a rather detailed analysis of floristic composition. The more prominent pasture types can generally be related from mere observation to the environmental factors which tend to produce them, but the conditions which give rise to less distinctive communities are seldom obvious, and require for their elucidation more extensive research than is possible in a primary survey.

Most important in determining the physiognomic type is the human factor, acting both directly and through grazing animals. Without it, grassland would not even exist in this part of the world, and its mode of operation decides how far pasture shall replace the native woodland. Grazing tends to produce a turf of low-growing, rhizomatous herbs, with growing points protected by being held close to the ground. A period of undergrazing allows the establishment of taller herbs, shrubs and trees. Or again, incomplete destruction of the woodland species when the land was first cleared may result in the persistence of some of these in spite of grazing. Thus, as shown in Table I there is found very commonly in our pastures not only (i) the turf layer of vegetation but also taller layers which are alien to such a community. These may be subdivided into (ii) tall herbaceous layer, (iii) shrub layer, (iv) tree layer. Except for an occasional shade tree, these last three layers are undesirable, and owe their presence, as previously mentioned, to imperfect operation of the human and herbivor factors. The abundant presence of

TABLE I

SCHEME OF CLASSIFICATION OF PASTURE TYPES

Physiognomic type: prominent vegetation layer	Moisture relation	Layer	Dominant species in each layer on brown forest soils
I Turf	*Hydrophytic* high water table	I	*Cyperaceae; Agrostis stolonifera*
		II	*Eupatorium perfoliatum*
		III	*Salix; Alnus*
II Tall herb	*Mesophytic* medium condition	I	*Agrostis alba; Poa pratensis; Trifolium repens; Festuca rubra*
		II	*Solidago; Dicksonia punctilobula*
III Shrub		III	*Spiraea; Crataegus; Betula* scrub
		IV	*Acer saccharum; Fagus grandiflora; Betula lutea*
IV Tree	*Xerophytic* dry knolls and slopes	I	*Danthonia spicata; Poa compressa*

one or other tall layer affords, however, an easily recognizable basis of primary classification of pastures and one which also conveys some idea of their economic value.

Next to the human factor, that which has most effect on the type of vegetation is the water supply. The grassland formation is typically mesophytic, but where the water table is high a sward with *Cyperaceae* dominant affords grazing of some value. On the other hand, on knolls and steep slopes, the rapid run-off causes the ground to hold less than its share of summer rain and to support only a drought-enduring vegetation of poor feeding value, such as *Danthonia*, and deep-rooted herbs. This effect, as Johnstone-Wallace (8) has shown, is largely obviated if only a good spongy turf is developed on such places. The height of the water table and the water-holding capacity of the soil are, in fact, everywhere important because of the long spells of summer drought during which water is the limiting factor in the growth of grassland vegetation.

Consequently a secondary subdivision of pasture types is made on this basis.

Further subdivision is made in terms of floristic composition, using any or all of the layers that may be present. Newton and Nowosad (9) and Newton and Stobbe (10) found the tree species to be most characteristic of the genetic soil type. Whyte, whose survey so far has been mainly confined to the brown forest soil, has concentrated on the turf and intermediate layers. The former, of course, is the important one. The factors, apart from water supply, which determine the dominant species of the turf are still obscure but it is believed that the general fertility level is of major importance. This may depend on a variety of conditions, notably the degree of leaching and podsolization, of soil erosion, of soil exhaustion, etc. In the primary survey

work, the botanical composition of the sward is arrived at either by eye observation and listing of estimated frequency, or by point quadrat readings. Analyses of typical swards are given in Table II.

TABLE II

POINT QUADRAT METHOD—"HITS" PER HUNDRED POINTS EXAMINED

	1*	2	3	4	5
Poa compressa	—	—	—	11	—
Festuca rubra	41	3	—	7	—
Agrostis alba	8	12	30	23	7
Poa pratensis	10	26	7	24	45
Trifolium repens	6	12	9	15	11
Achillea Millefolium	5	1	10	4	4
Phleum pratense	3	—	10	1	2
Viola spp.	3	—	2	1	—
Panicum lanuginosum	1	—	—	—	1
Fragaria virginiana	1	1	2	—	—
Hieracium aurantiacum	1	1	3	16	—
Taraxacum officinale	1	—	—	—	—
Cerastium vulgatum	1	3	—	—	—
Chrysanthemum Leucanthemum	1	—	4	—	—
Prunella vulgaris	1	2	4	—	—
Veronica serpyllifolia	1	3	—	—	1
Moss	8	3	3	—	—
Bare ground	26	27	22	14	34
Plantago major	—	1	—	—	—
Oxalis europaea	—	2	1	—	—
Plantago lanceolata	—	1	—	—	—
Aster spp.	—	1	—	—	—
Solidago spp.	—	1	—	—	—
Festuca elatior	—	—	—	—	2
Rumex Acetosella	—	3	1	1	4
Danthonia spicata	—	14	4	—	—
Hieracium florentinum	—	—	3	—	—
Carex spp.	—	—	3	—	—
Ranunculus acris	—	—	—	2	—

*1 *Festuca sward; Lawrenceville, July 1935.*
2 Poa : Danthonia, Agrostis, Trifolium; Dunham-Sutton Rd., June, 1935.
3 Agrostis sward; Dunham-Sutton Rd., June 1935.
4 Poa-Agrostis sward; Oak Hill, June, 1935.
5 Poa sward; Bull Pond, June, 1936.

The sward which is most general in the pastures of the Eastern Townships is one in which *Agrostis* spp. are dominant. Under mesophytic conditions, *Agrostis alba* is the prominent species. Here there may be an almost pure *Agrostis* sward, or a combination with *Trifolium repens* or *Poa pratensis*, or both. Generally speaking, *Agrostis alba* is believed to be indicative of a lower fertility level than is required by *Poa pratensis*. This is borne out by the fact that in the podsolized brown forest soils, *Agrostis alba* is the dominant species, while *Poa pratensis* although present does not become dominant. On the other hand, in paddocks where the cows are confined for milking, and in night pastures where the ground has received more than its fair share of droppings, *Poa pratensis* is seen to flourish (7). This dominance of *Poa pratensis* linked with abundance of *Trifolium repens* marks the best type of

pasture to be found in this area. Where hydrophytes prevail, *Agrostis alba* is also present, but *A. stolonifera* is more abundant. *Festuca rubra* presents a more difficult problem because of its sporadic occurrence. It may occur in swards covering large areas or in locally dominant patches. It has been found in comparatively wet areas but on the whole it favors drier places. It is found associated with *Agrostis alba* on the one hand and with *Danthonia spicata* on the other, and because of its distribution and associations, is presumably indicative of a fertility somewhat lower than that required by *Agrostis alba*. Of the other grasses occurring, only one is to be found in any quantity, *viz.*, *Danthonia spicata*. This grass inhabits dry places and pastures that have run out, and, as its name "Poverty grass" implies, it is a sure sign of a very low level of fertility. It is not relished by stock and is indeed a weed in the worst sense of the word.

Besides pastures in which the turf layer is most abundant, two other types are of quite common occurrence, namely those which consist largely of hardhack *(Spiraea tomentosa)* or of hay-scented fern *(Dicksonia punctilobula)*. Spiraea pastures occur widely in dry and sometimes in wet land, and are probably the result of undergrazing. *Dicksonia punctilobula* is also widespread, probably as a relic of the woodland flora. It grows in clumps and, while in a hardhack pasture there is usually some feed round the bushes, where *Dicksonia* grows, grass flourishes only in the spaces between the clumps. *Dicksonia* is thus a bad weed.

A much more detailed study of the turf layer in particular has been made by Dore (6) who has made use of the experimental fertilizer plots, referred to later in this paper, to determine the succession of grasses and clovers that takes place when the fertility factor is varied.

B. Natural Re-seeding

The natural re-seeding habits of plants associated with pastures is another ecological phase that has been given some attention. Dore (5) discusses the methods by which the flora of the turf layer provide for their maintenance. Propagation by vegetative means is by far the most important in permanent pastures. Some plants, of which *Trifolium repens* is an excellent example, increase by stolons. Others again have the underground rootstock or rhizome which is characteristic of *Poa pratensis*, *Agrostis stolonifera* and many others. The second important method of propagation is by seed, which may be distributed by natural means or transported by animals. Most of the seeds scattered by animals must possess resistance to the digestive processes.

In studying this question Dore collected samples of both soil and manure from old permanent pastures. Sods 6 × 6 × 1 in. were lifted in June and September from four pastures, and fresh manure droppings were obtained in early, middle and late summer from two areas. The samples were dried, broken up, mixed and sampled. Standard quantities were placed in flats of sterilized soil in the greenhouse to germinate. A duplicate set was subjected to a previous freezing and thawing to more nearly simulate field conditions.

Table III gives a list of the species found growing on the four untreated pastures worked with, together with their relative frequency expressed as percentage of ground covered.

TABLE III

PERCENTAGE GROUND COVERED BY SPECIES OF PLANTS ON UNFERTILIZED AREAS OF THE FOUR PERMANENT PASTURES IN THE VICINITY OF COWANSVILLE, QUE.

Species	Pasture				Average
	A	B	C	D	
Agrostis stolonifera	20.6	10.6	20.3	24.6	19.3
Festuca rubra	−	35.6	2.1	1.4	9.8
Hieracium aurantiacum	12.4	1.7	14.4	+	7.2
Trifolium repens	5.3	10.9	.8	9.4	6.6
Phleum pratense	8.7	2.1	9.5	4.4	6.2
Taraxacum officinale	6.7	1.2	+	13.3	5.3
Poa pratensis	4.0	2.0	+	6.7	3.2
Danthonia spicata	+	3.6	8.1	+	3.0
Plantago major	.8	.6	1.3	5.6	2.1
Ranunculus acris	2.1	.8	.5	5.0	2.1
Prunella vulgaris	2.4	1.4	1.3	2.1	1.8
Panicum lanuginosum	1.7	+	4.8	+	1.7
Fragaria virginiana	1.6	1.8	2.6	+	1.5
Carex spp.	3.2	+	1.5	.6	1.4
Oxalis europaea	1.1	2.2	1.4	+	1.3
Chrysanthemum Leucanthemum	+	1.9	1.8	+	1.1
Potentilla simplex	−	+	4.0	+	1.1
Cyperus diandrus	+	−	−	3.9	1.0
Solidago spp.	+	+	1.7	+	.7
Viola pallens	1.1	.7	+	.6	.6
Digitaria Ischaemum	+	2.5	−	−	.6
Hieracium florentinum	1.3	+	.5	−	.5
Achillea Millefolium	+	1.7	+	+	.5
Juncus macer	.9	+	+	.7	.4
Antennaria neglecta	.7	.7	+	+	.4
Linum catharticum	−	−	−	1.4	
Poa compressa	.5	+	+	.6	
Glyceria striata	.5	−	−	+	
Stellaria graminea	+	−	.6	−	
Trifolium agrarium	−	+	.7	+	
Cerastium vulgatum	+	+	+	+	
Veronica serpyllifolia	+	+	+	+	
Cirsium spp.	+	−	+	+	
Hedeoma pulegioides	+	−	−	−	
Lycopus spp.	+	+	+	+	
Oenothera pumila	+	+	+	+	
Potentilla norvegica	+	+	+	+	
Sisyrinchium angustifolium	+	+	+	+	
Spiraea tomentosa	+	+	+	−	
Sporobolus neglectus	+	−	−	−	
Vicia Cracca	−	−	+	−	
Trifolium pratense	−	+	+	+	
Leontodon autumnalis	+	+	−	+	
Rumex Acetosella	+	+	+	+	
Hypericum spp.	+	+	+	+	
Festuca elatior	+	+	+	+	
Hydrocotyle americana	+	+	+	+	
Agropyron repens	+	−	+	−	
Equisetum arvense	+	−	−	+	
Echinochloa crusgalli	+	−	−	−	
Erigeron sp.	+	+	+	+	
Galium sp.	+	+	+	+	
Lobelia inflata	+	+	−	−	
Plantago lanceolata	−	+	−	−	
Veronica officinalis	−	+	−	−	
Setaria lutescens	−	−	+	−	

Plants present in less than 0.5% of ground cover are indicated by +.

The data obtained from the soil samples are presented in a very brief way in Table IV. This shows an average for the district of 77 million potential plants per acre, which represents more than four times the number of actual plants ordinarily found growing in a permanent pasture. The seeds were, for the most part, from plants that were not very important in the sward. Five species that contributed 63% of the seed represent less than 7% of the sward.

TABLE IV

THE MORE IMPORTANT SEEDLINGS ARISING FROM VIABLE SEEDS IN SOIL FROM PERMANENT PASTURES

Species	Number of seedlings per sq. ft. of pasture surface				Ave. no. per acre, millions
	A	B	C	D	
Cyperus diandrus	56	—	—	366	18.38
Juncus macer	88	15	97	85	12.41
Danthonia spicata	—	108	39	1	6.45
Chrysanthemum Leucanthemum	1	2	115	—	5.14
Cerastium vulgatum	16	—	28	51	4.15
Panicum lanuginosum	21	—	59	—	3.48
Agrostis stolonifera	28	7	24	10	3.01
Plantago major	14	1	2	40	2.48
Potentilla norvegica	6	16	19	15	2.44
Erigeron ramosus	—	—	54	1	2.40
Hypericum spp.	31	—	4	14	2.13
Digitaria Ischaemum	—	31	—	8	1.70
Lobelia inflata	9	9	1	12	1.35
Oenothera spp.	6	1	17	5	1.26
Poa compressa	13	4	—	7	1.05
Forty-four others in smaller amounts					
Total number of seedlings:	342	218	537	675	77.19
Number of species	32	21	33	32	57

The manure samples examined presented quite a different situation. From the standpoint of the species involved, livestock represents the most important seed distributing agency. Each mature cow was shown to distribute, on the average, more than one and one-quarter million seeds during a single grazing season. Table V gives a much curtailed picture of the results obtained. Five of the most valuable pasture plants contribute 70% of the seed. As would naturally be expected, a high correlation was found between the seasonal flora and seeds present in the manure samples.

Dore's data show an enormous potential supply of new plants available to the pasture sward. Under suitable conditions this supply may become a very potent factor. Results both here and elsewhere, however, leave little doubt that vegetative development is mainly responsible for the multiplication of the characteristic herbage species typical of a permanent pasture, when favorable conditions for that development are provided.

TABLE V

THE MORE IMPORTANT SEEDLINGS ARISING FROM VIABLE SEEDS IN MANURE FROM PERMANENT PASTURES AT PERIODS DURING THE SEASON

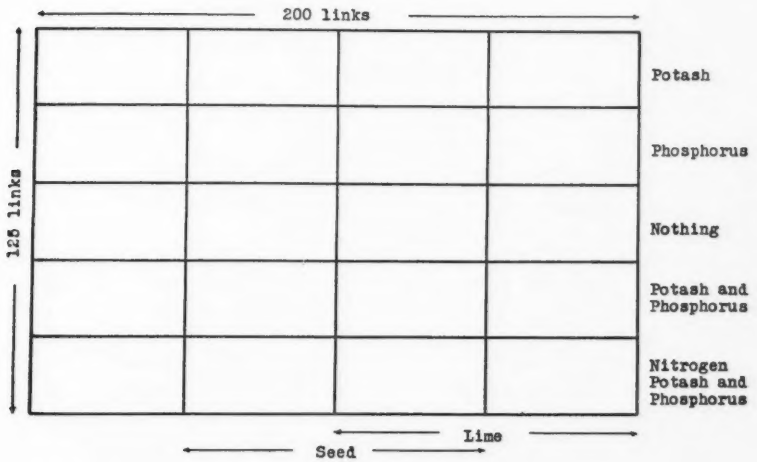
Species	Number of seedlings per 10 ounces of dried cattle manure						No. distributed by cow in 165 days, thousands
	Pasture B			Pasture D			
	June 20	Aug. 6	Sept. 10	July 20	Aug. 15	Sept. 10	
Agrostis stolonifera	—	237	118	259	91	44	291.34
Phleum pratense	18	39	347	25	47	55	206.54
Poa pratensis	1	199	12	132	17	29	151.69
Trifolium repens	3	161	25	67	42	19	123.30
Chenopodium album	—	—	245	—	—	6	97.63
Poa compressa	2	65	24	85	33	28	92.18
Cerastium vulgatum	65	15	2	7	5	—	36.56
Carex spp.	—	7	14	51	19	—	35.40
Plantago major	2	2	64	3	5	15	35.40
Veronica serpyllifolia	57	2	4	2	—	—	25.28
Danthonia spicata	5	11	15	17	4	—	20.23
Ranunculus acris	—	4	14	9	6	—	12.84
Plantago lanceolata	—	7	7	15	—	—	11.28
Thirty-six others in smaller amounts							
Total number of seedlings	167	795	970	719	291	212	1228.70
Number of species	13	26	33	27	19	19	49

III. Location and Experimental Procedure

Reference has already been made to the association of these investigations with the brown forest soil type. Representative farms were chosen centering about the town of Cowansville, Que. The initial procedure was concerned largely with the establishment of a satisfactory technique. While seeking possible means of improvement it was recognized that a typical pasture environment must be maintained. Seven farms were originally selected and surface applications of the standard fertilizers with and without lime and seed were given. Fig. 2 shows the arrangement of the first experimental areas. Weaknesses fairly soon became apparent in this method as the response to the mineral and complete fertilizers was so outstanding that the plots were almost immediately subjected to gross overgrazing. This difficulty was overcome in subsequent years by fertilizing from three to five acres surrounding the trial section and the plan of the experiments has been radically altered to measure as well the rates of the minerals. Randomization of the various treatments has also been resorted to. The standard plan at present in use is shown in Fig. 3.

Yields have been taken each year from the treated plots through the use of a relatively inexpensive wire cage which covers exactly a square yard. Clippings are made four times during the season, the cage being moved each time to a fresh area. The clipped samples are dried to constant weight with as little delay as possible.

Plan of Series I Pasture Plots



Rate of Fertilizer Application:

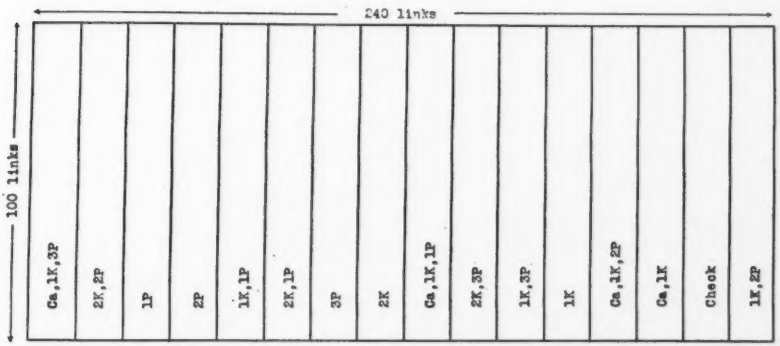
Nitrate of Soda	- 200	lbs.
Superphosphate 16%	- 500	"
Muriate of Potash	- 160	"
Ground Limestone	- 4000	"

Seed Mixture:

Kentucky Blue	Meadow Fescue
Canada Blue	Red Fescue
Red Top	Awnless Brome
Orchard Grass	White Clover

FIG. 2. *Plan of early plot experiments used in connection with this project.*

Plan of Series V Pasture Plots



Key to Treatments:

1P = 300	lbs.	Superphosphate 20%
2P = 500	"	" "
3P = 700	"	" "
1K = 100	"	Muriate of Potash
2K = 200	"	" " "
Ca = 4000	"	Ground Limestone

Systematic Arrangement of Plots:

1. Check	7. 1K,2P	13. CalK
2. 1P	8. 1K,3P	14. Ca,1K,1P
3. 2P	9. 2K	15. Ca,1K,2P
4. 3P	10. 2K,1P	16. Ca,1K,3P
5. 1K	11. 2K,2P	
6. 1K,1P	12. 2K,3P	

FIG. 3. *The standard type of plot experiment now employed.*

Careful record has been made of the floral change resulting from the treatments given. The grid quadrat was used for this purpose, the results being recorded as percentage of ground covered by the various species. The standard size now in use is 50 × 50 cm. divided into the usual 25 squares of 10 cm.

Inasmuch as a great many of these data have already been published (6, 11) no detailed reference will be made to yield or succession data in this paper.

IV. Chemical Phases—Phosphorus Studies

During the past five years yield data have been collected from a large number of plots located on representative brown forest soils. The results obtained on these soils show that, among the elements applied as chemical fertilizers and amendments, phosphorus is outstandingly the most significant in increasing the yields and quality of pasture herbage. Table VI A, from Stobbe (15), shows the effect of phosphate fertilization on the yield of the clipped herbage. The B. section of the Table records the effect of phosphorus on the nitrogen (N_2) content. From the succession studies it is apparent that much of this increase is an indirect one resulting from the material increase in the clover fraction of the clippings. The effect on the phosphorus content of the clipped herbage due to applications of superphosphate is shown in the C. portion of Table VI. This again shows a marked increase in the phosphorus (P) on plots that were treated with that element. This result probably is indirect to some extent as well.

The highly significant action of phosphorus has led to a study of its soil and plant relationships.

TABLE VI

A. EFFECT OF PHOSPHATE FERTILIZATION ON THE YIELD OF HERBAGE CLIPPED FROM PLOTS, 1931
(6 farms, 24 plots per treatment)

Treatments with P	Total weight of clipping	Treatments without P	Total weight of clipping
CaPK	2642	CaK	1767
PK	2427	K	1473
CaP	2302	Ca	1611
P	1966	Nil	1425

B. EFFECT OF PHOSPHATE FERTILIZATION ON THE NITROGEN CONTENT OF HERBAGE CLIPPED FROM PLOTS, 1931
(6 farms, 24 plots per treatment)

Treatments with P	Average % N_2 in herbage	Treatments without P	Average % N_2 in herbage
CaPK	3.44	CaK	2.74
PK	3.37	K	2.56
CaP	3.14	Ca	2.53
P	2.87	Nil	2.52

C. EFFECT OF PHOSPHATE FERTILIZATION ON THE PHOSPHORUS CONTENT OF HERBAGE CLIPPED FROM PLOTS, 1932
(6 farms, 24 plots per treatment)

Treatments with P	Average % P in herbage	Treatments without P	Average % P in herbage
CaPK	0.338	CaK	0.279
PK	0.334	K	0.282
CaP	0.327	Ca	0.292
P	0.345	Nil	0.295

A. Method of Extracting Available Soil Phosphates

The currently used methods of extracting soil phosphates for study have not been conceived primarily from the standpoint of soil conditions. Russell (14) shows that the soil drainage water, obtained through drainage, not run-off, contains a high proportion of calcium among cations, and sulphate among anions. Analytical figures for the water solutions from North American soils show similar high proportions of lime and sulphate sulphur in the total solids. Wrenshall and McKibbin (17) have employed a solution, the use of which is termed the "Quebec" method, for the extraction of phosphate phosphorus in which calcium is the dominant cation and sulphate the dominant anion. The solution is made up to have a reaction value of pH 3.0. Results are given showing that, by this method, the extracted phosphates conform more closely to the plant growth response.

B. Penetration of Applied Phosphorus

Samples of permanent pasture soils both treated and untreated have been collected and extracted to determine the vertical distribution of the phosphate phosphorus which is available to plants. The samples taken were the upper half-inch, the next inch and the next one and one-half inch. Treatment consisted of 700 lb. of 16% superphosphate applied as a surface dressing. The soils were sampled approximately four months after the treatment was given. Table VII shows the amount and location of the total and available phosphates under the two conditions. Much the greater part of the applied phosphorus is retained in the upper half-inch of the soil.

TABLE VII

VERTICAL DISTRIBUTION OF PHOSPHORUS IN FERTILIZED AND UNFERTILIZED BROWN FOREST PASTURE SOIL

Depth of soil sample	p.p.m. of P in air dry soil		
	Unfertilized		Fertilized
0 – ½ in.	Total	1000	1200
	Available	12	80
½ – 1½ in.	Total	850	880
	Available	8	8
1½ – 3 in.	Total	700	700
	Available	7	7

C. Depletion of Phosphorus by Cropping

In Table VIII data are presented showing the available phosphate phosphorus of a fertilized soil one, two, and three years after application of 500 lb. per acre of 16% superphosphate to the surface.

TABLE VIII

DEPLETION OF AVAILABLE PHOSPHORUS FROM SURFACE HALF-INCH OF BROWN FOREST PASTURE SOIL

Designation of sample	Year of fertilization	No. crops removed since fertilization	Available P in p.p.m. of soil at Sept. 1934
D_4PA	1934	1	53
D_3PA	1933	2	33
D_2PA	1932	3	12
D_2NA	–	–	6

It will be noted that the available phosphorus rapidly decreases annually, and after three years closely approaches the original content.

D. Pot Cultures with Brown Forest Soils

In order to study further, under close control, the response of pasture plants to soil conditions, 78 pot cultures were established in the greenhouse. The soil used was a typical brown forest soil under permanent pasture. The two indicator plants chosen were wild white clover and timothy, and each of them was propagated clonally for this trial. The former in particular has, in the field, given very striking response to phosphate fertilization in Quebec, New York state and in the British Isles. The experiment is not yet complete but in Table IX results are presented for the first two clippings illustrating the effects of lime and sulphur applied to the soil alone and with added phosphate phosphorus.

TABLE IX

A. TOTAL YIELDS OF TIMOTHY AND CLOVER TAKEN FROM TREATMENTS IN FIRST TWO CLIPPINGS
(Pot cultures)

Treatment, pounds per acre		Yield in grams
Nil		11.0
Limestone	2000	9.0
Sulphur	150	13.1
Superphosphate	300	13.8
Superphosphate + limestone	300 + 2000	12.0
Superphosphate + sulphur	300 + 150	14.5
Superphosphate	700	15.7
Superphosphate + limestone	700 + 2000	15.0
Superphosphate + sulphur	700 + 150	18.4

B. PHOSPHORUS UPTAKE FROM TREATMENTS BY GROWING TIMOTHY AND CLOVER
(Pot cultures)

Treatment, pounds per acre		Milligrams of P removed by plants
Nil		11.5
Limestone	2000	9.5
Sulphur	150	15.8
Superphosphate	300	19.2
Superphosphate + limestone	300 + 2000	14.7
Superphosphate + sulphur	300 + 150	18.9
Superphosphate	700	24.2
Superphosphate + limestone	700 + 2000	20.8
Superphosphate + sulphur	700 + 150	26.7

It will be seen that lime, which lessens soil acidity, exerts a repressive effect on phosphorus uptake by plants and on the dry matter in crop yields, while elemental sulphur, which increases soil acidity, has increased both phosphorus uptake by plants and crop yields in dry matter. It is interesting to note that these facts coincide with observed, but unpublished, field results obtained in Quebec with lime and sulphur on pasture soils of a similar character. The clover and timothy independently show these responses.

E. Nature of Organic Phosphorus in Soils.

Phosphorus in Quebec pasture soils is usually present in considerable quantity, but not, however, in a form readily available to plants. The nature of soil inorganic phosphorus is fairly well known. A laboratory study is being made of the molecular forms in which this element in organic combination exists in our soils. This investigation is still in progress but is sufficiently advanced to permit the presentation of some results relevant

to the organic fraction. Two soils have been worked with, a typical brown forest pasture soil and a black muck soil, high in organic matter. In the former, 30% of the phosphorus was in the organic form and 70% inorganic, while in the muck soil 55% was organic and 45% inorganic. The most complete study, to date, has been made of the black muck soil. It was high in phosphorus to begin with, having a total phosphorus (P_2O_5) content of 0.5%. Of the total organic phosphorus present less than 0.5% was found to be lecithin and somewhat more than 65% was nucleic acids. The remaining portion has not yet been identified but may also be, at least in part, nucleic acids. The method of isolation and purification of soil nucleic acids has been revised and improved.

V. Nutritional Studies

In 1930 a series of three grazing trials was outlined, two trials with dairy cattle and one with beef steers, with the object of measuring the effects of mineral fertilization of natural pastures on their stock carrying capacity. The dairy cattle trials were discontinued after the first year, but the steer grazing tests have been continued. The results of the first four years of this project (2) indicated a very marked increase in steer carrying capacity resulting from the treatments given. Only one season's results are thus far available from the test now in progress. These show an increase of some 57% in total weight of beef produced per acre and 75% in steer days of grazing creditable to fertilization.

It was felt, however, that while grazing trials gave certain useful information, they could not be expected satisfactorily to measure the nutritive value as distinct from yield of pasture herbage. It was also felt that the nutritive value or feeding value per unit of weight of feed might in many cases be of as much importance in explaining the results of feeding tests as yield of herbage.

In 1933 it was decided to undertake studies aimed at the evaluation of the nutritive value of pasture herbage through the controlled feeding of clippings. Any such plan automatically eliminated the possibility of using the larger farm animals in the feeding tests. The large quantities of clippings which would be required for such stock could not be arranged for under our conditions. Thus some laboratory animal seemed the only solution, and rabbits, because of the similarity of their natural diets to those of farm ruminants, were chosen. No data were available to us as to the technique of laboratory management of rabbits for tests in which feed consumption records were essential, nor were specifications of suitable hutches or feeding equipment to be had. The first step in the project was therefore one largely of technique, which took the most of our first season's feeding work. Our present equipment is very satisfactory for feeding and metabolism tests and, with normal diets, feed loss is reduced to something under 1% of the total allowance, which represents a very small experimental error as compared with other sources of error in such tests with any class of stock.

Preparation of Diets

The procedure followed in the collection and preparation of the diets has been practically the same throughout the work thus far. For the mixed herbage from actual pasture fields, the plan has been as follows: A reasonably smooth area in a pasture field is selected and fenced, and about one-third of it is treated with mineral fertilizer at the rate of 500 lb. superphosphate (16% P_2O_5) and 100 lb. muriate of potash per acre. This is done in the fall where possible. The next pasture season the areas are periodically mowed with a lawn mower, the aim being to clip the herbage frequently enough to keep it in about the stage of maturity that is found in grazed pasture. This usually means a clipping once a month, or perhaps oftener in the spring. In general the herbage will be cut when less than four inches in height. These clippings are dried in the sun, coarsely ground, and stored in metal containers for feeding.

In addition to the clippings from actual pastures, feeding tests have been made on samples of pure species of different grasses grown on plots of the Agronomy Department. These have been treated in the same manner as described for the mixed herbage clippings.

It might be stated here that it is recognized that herbage clippings cannot be considered to represent in every respect the material eaten by grazing animals. Selective grazing is not imitated by this method, and while in good pastures this feature may not be an important factor, it becomes increasingly of significance in cases where the mixed herbage contains appreciable amounts of material avoided by the animal. Further, sun drying in all probability results in the leaching of some nutrients and possibly in alteration or destruction of others (vitamins). This latter problem is not as serious within a trial as might be thought, since all diets have received the same preparation, and hence may not seriously complicate comparisons. In this respect it may be pointed out that the chemical analyses of the diets fed over the past three years fail to show changes indicating any marked effects traceable to this method of preparation which would interfere with comparisons made.

In trials at present under way, clippings are being taken from areas containing almost no other grasses than bluegrass and redtop and these are being artificially dried as they are clipped. Thus the problems presented by selective grazing, in so far as it concerns weeds and objectionable grasses, and by the changes from sun drying and exposure, are minimized.

Source of Rabbits

Excepting for one test, the rabbits used have been bred and raised at Macdonald College. The breeding does trace to two white segregates of the New Zealand Red breed purchased in 1930. The females now in the colony are either half or full sisters or mother and daughter in relationship. Test rabbits in any one season are all by the same buck. This breeding plan has given satisfactory uniformity in the test stock. In general the litters have been weaned at about seven weeks of age and the young rabbits put on test

rations within the next two or three weeks. The tests have been standardized to a seven-day preliminary period followed by a 28 day test period. For the most part the feeding groups in any one test have contained five rabbits.

Results of Feeding Tests

To review in detail the 35 standard feeding groups together with the numerous special studies which have been completed since this work was started is out of the question in this paper. Some of the findings have already been published (3, 4), while more recent ones have been summarized by Cameron (1).

Taking the data of all the standard tests over the three years one feature stands out above all others, namely that results (gains of rabbits) have not been duplicated from one year to another, though replicate tests using the same diets have yielded comparable findings. In particular it has been evident that the herbage of different years has shown markedly different levels of nutritive worth. In view of the widely different conditions of moisture and temperature during the three years involved, this would perhaps not be surprising, were it not for the fact that the chemical composition (usual feeding stuffs analysis) of the herbage samples has shown relatively little variation, as compared with that of the gains made.

Some idea of the variability between lots may be had from Table X. Lot averages for the 35 tests were used as single observations as the basis for these calculations.

TABLE X

STANDARD DEVIATIONS OF LOT MEANS

Variable	*n*	Mean	Standard deviation	Coefficient of variation
Gain—28 days	35	94.9143	198.06	208.67
Daily feed	35	110.7143	20.57	18.6
Initial weight of rabbit	35	1,366.3428	178.25	13.1
% Crude protein	35	15.8629	3.08	19.4
% Crude fibre	35	23.1429	2.46	10.6
% N-free extract	35	49.4286	3.33	6.7

It may be pointed out that the chemical data represent the diets as fed and hence include cases in which supplements such as casein or sugar were involved. These often altered the protein or fibre levels of the rations. Hence the variability of the protein and fibre values is larger than that for the herbage clippings alone.

Simple correlation studies of the data indicate no significant relations between gains and any other of these variables excepting fibre. Here the relation is negative ($r = -.5039$).

An analysis by the method of partial regressions in which the dependent variable was 28-day gain and the independent variables were daily feed, initial

weight of rabbits, percentage of protein, percentage of fibre, and percentage extract, shows very clearly that percentage fibre is the most important of N-free these factors influencing gains of the rabbits, and percentage of protein the least. Taken together these five variables account for but 38.4% of the variability of the gains, which of course means that other important factors are involved. In this connection an earlier report (3) suggested quality of protein as one possible factor. In any case it seems certain that quantity of protein was not a factor in these tests.

Percentage of fibre in the diet appears to be an item of some significance. It is entirely probable, however, that the nature of the fibre is of greater importance than the total amount. This is suggested by Woodman (18) and is further indicated in these data by the fact that the lots which show the greatest gain are those (i) in which the fibre has been reduced by dilution of the diet with a fibreless material such as sugar, or (ii) in which wild white clover was the herbage and in which a particularly small amount of stemmy material was present. Delete these cases and the correlation between amount of fibre and gain drops below significance.

The chief conclusion to which these data point is that the ordinary feeding stuffs analysis may be of questionable value in predicting the nutritive value of pasture herbage. This is especially true with percentage of crude protein and suggests that care should be taken in pointing to changes in protein level of the herbage as justification for fertilization or other pasture treatment.

Acknowledgments

The author wishes to acknowledge the assistance received in the preparation of this paper, from Dr. G. W. Scarth and Messrs. W. G. Dore and J. H. Whyte in the ecological aspects of the work, from Dr. R. R. McKibbin in the soil and chemical phases, and from Prof. E. W. Crampton in the nutrition work.

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VIRUS STUDIES

I. THE PRODUCTION OF ANTISERA IN CHICKENS BY INOCULATION WITH POTATO X[1]

By W. Newton[2] and H. I. Edwards[3]

Abstract

Chicken antiserum was produced by three wing vein inoculations with sap from *Datura meteloides* and *Datura Stramonium* plants infected with "potato virus X". Before injection, the saps were purified by the Bawden and Pirie method. This antiserum formed a conspicuous precipitate when incubated for three hours at 37° C. with similarly purified sap of these two plant species when they were infected with the X or healthy potato virus, but failed to form any precipitate when incubated in the same way with purified sap from virus-free plants. Two unknown viruses, one from spinach and the other from tomato were established as belonging to the X group by the precipitin reaction through the use of chicken antisera. The serological grouping was supported by the fact that the unknowns had similar, if not identical lethal temperatures, longevities in vitro, and host ranges as the ordinary potato virus X.

Introduction

The specificity of the antisera secured from rabbits inoculated with the sap of virus-infected plants has assisted materially in the classification of plant viruses. In the case of plants infected with the ordinary tobacco mosaic virus, the formation of precipitate through the union of sap and antiserum is independent of the plant host from which the virus-infected sap is derived, at least within the family *Solanaceae*, according to Beale (3, 4), Birkeland (5), and Chester (6). If the precipitin reaction is independent of the host, as all evidence to date seems to indicate, then serological methods will remove much of the confusion that has arisen in the classification of plant viruses through the existence of the same virus or strain of the same virus in distinct hosts. All investigators agree that two or more viruses may be quite distinct as far as symptom expression in specific hosts is concerned, yet may be serologically identical; hence the precipitin reaction will serve to establish fundamental groups, but other means may have to be employed to identify virus strains within a serological group.

Up to the present rabbits appear to have been used almost exclusively in the study of plant viruses. It may be of interest that chickens respond in a similar way to injections of purified plant saps infected with a specific virus.

Experimental

The saps from *Datura meteloides* and *Datura Stramonium*, respectively, infected with potato X, the healthy potato virus, were purified according to the method described by Bawden and Pirie (1). Three wing vein injections of 1.5 cc., 2 cc., and 2 cc. respectively were made at three day intervals into

[1] *Manuscript received September 28, 1936.*
Contribution No. 463 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
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mature White Wyandotte chickens. Eight days after the last inoculation the blood samples were drawn. The serum from these blood samples produced conspicuous precipitates when incubated for three hours at 37° C. and cooled overnight at 7° C. with the saps of X-infected plants but produced no precipitates when incubated with saps similarly purified, but free from X virus infection. Two tobacco ring spot viruses, one originally isolated from spinach and the other from tomato, reacted positively with chicken antisera. Although these viruses proved to be distinct from each other and from the strains of the X virus so far isolated by us from potatoes, as judged by their symptom expression on several hosts, the fundamental properties of all three viruses, such as lethal temperature, longevity *in vitro*, and host range, were very similar if not identical. The precipitin reaction with chicken antisera has established them as belonging to the X virus group.

TABLE I

PRECIPITIN REACTION OF CHICKEN ANTISERA AND PURIFIED PLANT SAPS

Sap from	Virus present	Dilution of antigen (purified sap)			
		1 : 1	1 : 10	1 : 20	1 : 200
Datura meteloides	"X"	++++	+++	++	±
Datura meteloides	None	0	0	0	0
Datura Stramonium	"X"	++++	+++	++	±
Datura Stramonium	None	0	0	0	0
Nicotiana tabacum (White Burley)	Unknown virus from tomatoes	++++	+++	++	±
Datura Stramonium	Unknown virus from spinach	++++	+++	++	±

Discussion

Only two chickens provided the antisera used in these experiments but samples from both reacted specifically with three forms of the X virus; at least no visible precipitate could be seen when similarly purified but virus-free saps were incubated with either sample of antisera. The failure to detect any differences in the antisera reaction between different forms of the X virus and the entirely negative results with virus-free saps are in agreement with those of Bawden (2) who studied the antigenic properties of the X virus by means of rabbit antisera. It would appear that the antigen prepared by us from virus infected plants cannot be diluted to the same extent as the antigen used by Chester (7) against rabbit antisera. As will be seen from Table I, when the virus antigen was diluted to 1 : 200 the amount of the precipitate formed was so slight that it was barely detectable. A comparative study of chicken and rabbit antisera will be reported later.

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VIRUS STUDIES

II. STREAK X, A DISEASE OF TOMATOES CAUSED BY A VIRUS OF THE POTATO X GROUP UNASSOCIATED WITH TOBACCO MOSAIC[1]

By William Newton[2]

Abstract

A streak disease of tomatoes was found to be caused by a virus of the potato X group unassociated with tobacco virus 1. The disease markedly reduced the yield of marketable fruit in several greenhouses near Victoria. The symptoms resemble those induced by ordinary potato virus X in conjunction with tobacco mosaic. The host range, lethal temperature, longevity *in vitro*, and dilution extinction point of the virus resemble ordinary potato X. Streak X may be distinguished from ordinary potato X by the more pronounced symptoms it induces on tobacco, *Datura*, *Nicotiana glutinosa*, and tomato, and particularly by the streaking and necrosis of the stems and leaves of tomato. The virus causing this streak disease could not be recovered from Irish Cobbler potatoes after an incubation period of ten days, neither did the characteristic symptoms occur on tomatoes already infected with the ordinary potato virus X. The virus was recovered unchanged from X-free potato seedlings. The antigen reaction also proved that the streak virus belonged to the potato virus X group.

Introduction

A disease of tomatoes caused by a virus of the potato X group unassociated with tobacco mosaic was found in several greenhouses near the city of Victoria, British Columbia. A pronounced striping and necrosis of the stems and leaves and a blotching of the fruit were the characteristic symptoms. In several houses more than 50% of the plants were infected, and from the infected plants, little marketable fruit was secured. The characteristic symptoms resemble those of so-called "experimental streak", the disease which appears when tomato plants that are infected with tobacco virus 1 are re-inoculated with the potato virus X.

Experimental

Streak X was transmitted to tobacco (White Burley), *Datura Stramonium*, *D. meteloides*, *Nicotiana glutinosa*, and tomatoes by rubbing the leaf surfaces with a glass spatula moistened with the sap from infected tomato foliage. The information with respect to symptom expression is summarized in Table I

TABLE I

The symptoms induced by streak X and potato X on specific hosts

Virus	Tobacco (White Burley)	*Datura meteloides*	*Datura Stramonium*	*Nicotiana glutinosa*	Petunia	Tomato
Streak X	RMn	rMn	rMN	MN	0	MNS
Potato X	rm	rmn	m	m	0	m (faint)

Explanation of symbols: r = *rings*, m = *mottle*, n = *necrosis*, s = *streaks, and* 0 = *no visible symptoms. Capitalization indicates that the symptom is very pronounced.*

[1] *Manuscript received September 28, 1936.*
Contribution No. 464 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
[2] *Pathologist-in-charge, Dominion Laboratory of Plant Pathology, Saanichton, B.C.*

and is contrasted with the symptoms induced by a local form of the potato virus X isolated by inoculating *Datura Stramonium* with the sap of locally grown Irish Cobbler potato tops. It will be noted that the host range is similar, but in all cases the symptoms induced by streak X are more severe than those induced by potato X from Irish Cobbler potatoes. On tobacco plants inoculated with streak X, conspicuous multiple rings appeared as both primary and secondary symptoms. However, the secondary symptoms were more frequently a net-like mottle followed by considerable necrosis. Potato X on tobacco sometimes appeared as faint rings but more frequently as a mild mottle only. On *Datura Stramonium* the mottle induced by streak X was rapidly followed by necrotic lesions. Under the same conditions, potato X induced a mottle only. On *Datura meteloides*, the symptom expressions of the two viruses were practically identical. Those induced by streak X appeared in a shorter time and were slightly more pronounced. On *N. glutinosa*, the mottle was followed by a pronounced necrosis in the case of streak X but a mottle only appeared when this species was inoculated with potato X. On tomatoes inoculated with streak X, a blotchy mottle first appeared, followed by streaks of a purplish tinge on the leaves and broad streaks of a similar color on the stems. These streaks later became necrotic and brown. Potato X induced on tomatoes a faint and barely detectable mottle only.

The absence of the characteristic local lesions of tobacco virus 1 on *D. Stramonium* and *N. glutinosa* proved that this disease was distinct from any form of the "streak group" described by Ainsworth (1).

No significant difference was found in the lethal temperature, longevity *in vitro* or dilution extinction point of streak X compared with potato X, as may be seen by examining the data in Tables II, III, and IV. Their similarity is suggestive that the virus causing the tomato streak X belongs to the potato virus X group. The lethal temperature of the virus causing this disease is between 65° and 70° C., hence tobacco virus 1 is not involved in this disease as in the case of "experimental streak."

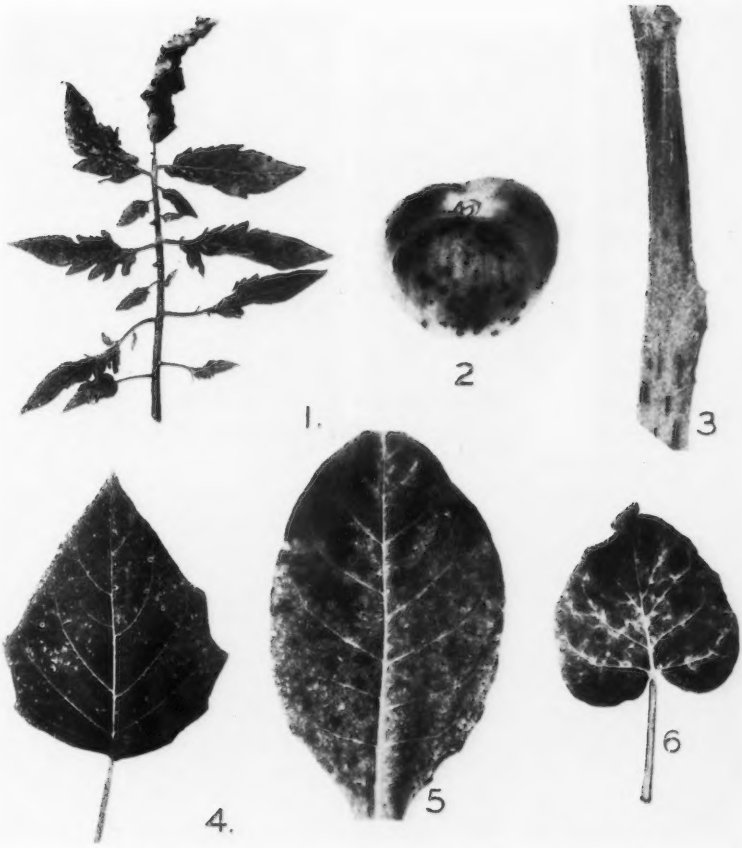
TABLE II

THE LETHAL TEMPERATURES OF STREAK X AND POTATO X IN *D. Stramonium* SAP DILUTED 1 : 20

Virus	Exposed in thin walled tubes for 10 min.				
	60°	65°	67°	69°	71°C.
Streak X	15*/15	15/15	15/12	15/2	10/0
Potato X (Irish Cobbler)	15/15	15/15	15/11	15/0	10/0

* *Number of plants inoculated over the number that became infected.*

PLATE I



Symptoms of streak X on tomato. (1) Leaf mottle and necrosis. (2) Fruit blotch. (3) Stem streaks. (4) D. meteloides, mottle and necrotic spots. (5) On tobacco, multiple rings. (6) On N. glutinosa, pronounced mottle.

TABLE III

THE LONGEVITY OF STREAK X AND POTATO X IN *D. Stramonium* SAP DILUTED 1 : 100 AT ROOM TEMPERATURE (APPROXIMATELY 20° C.)

Virus	Days					
	1	4	8	12	16	20
Streak X	5*/5	5/5	5/5	5/5	5/2	5/0
Potato X (Irish Cobbler)	5/5	5/5	5/5	5/5	5/0	5/0

* *Number of plants inoculated over the number that became infected.*

TABLE IV

THE SAP DILUTION EXTINCTION POINTS OF STREAK X AND POTATO X

Virus	Dilution of infected *D. Stramonium* sap				
	10^2	10^3	10^4	10^5	10^6
Streak X	15*/15	15/15	15/15	15/8	15/1
Potato X	10/10	10/10	10/10	10/2	10/0

* *Number of plants inoculated over the number that became infected.*

Thung (6), Salaman (4), Kunkle (2) and others have shown that plants cannot be inoculated with a virus that induces marked symptoms if already infected with one that induces little change from normality if both belong to the same group. Although potato X from local Irish Cobbler induces little change in tomato plants, the presence of this virus completely immunized the tomato plants against streak X. Ten days after inoculating plants with potato X they were re-inoculated with streak X. No symptoms subsequently developed beyond the mild mottle characteristic of potato X. Furthermore, when apparently healthy Irish Cobbler potatoes were inoculated with streak X, ten days later only the ordinary potato X could be recovered by transferring the sap to *D. Stramonium* and tomatoes. On the other hand, when X-free potato seedlings were inoculated with streak X, ten days later transfers of the seedling sap induced the characteristic streak disease on tomatoes and the characteristic mottle and necrosis on *D. Stramonium*.

The precipitin reaction proved also that the virus of streak X belongs to the potato virus X group (3).

Discussion

The discovery of an important streak disease of tomatoes caused by a virus of the potato X group, unassociated with tobacco virus serves to emphasize the economic importance of distinguishing between the virus types or strains

within a group as established by serological and protective inoculation means. It would appear from this study that the ordinary form of potato X is not pathogenic in tomatoes except when associated with "tobacco virus 1", but that forms of the X virus exist that can cause a serious disease of tomatoes. Salaman and his associates (5) have demonstrated that the X virus in potatoes may be a mixture of types. They were able to break up the original X as isolated from potatoes into distinct forms. It would be interesting if a form exists in potatoes that is capable of inducing conspicuous necrosis in tomato. This study indicates that X-free potato seedlings in conjunction with potato plants of any American variety are of value in demonstrating whether a virus of the potato X group is present in a plant other than potatoes. Any form of X may be recovered unchanged from potato seedlings, but from commercial potatoes only the form of X already present can be recovered.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 14, SEC. D. NOVEMBER, 1936 NUMBER 11

STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

IV. FURTHER PARASITES FROM TRINIDAD DEER[1]

BY THOMAS W. M. CAMERON[2]

Abstract

The trematode, *Paramphistomum cotylophorum* is recorded from *Mazama simplicicornis*, and the female of the nematode *Eucyathostomum longesubulatum* is described.

Since the publication of my last paper on the parasites of the Trinidad deer, *(Mazama simplicicornis)* Professor F. W. Urich has collected five other specimens of this host. These were fixed in formalin in the usual manner but only the intestines and liver were sent to the Institute for examination. Seven species of helminths—one trematode, one cestode and five nematodes—were recovered; all except one being found in the intestine.

Paramphistomum cotylophorum FISCHOEDER, 1901

(SYN: *Cotylophoron cotylophorum*)

This trematode was found in some numbers in two of the deer, in both cases in the duodenum. Most of the specimens were minute, but some were fully developed and contained a small number of eggs; none however had reached its maximum size. Even in the very small specimens in which the genitalia was still a rudiment, the genital sucker was visible and conspicuous. The larger specimens were sufficiently mature to show the excretory canal crossing the Laurer's canal and to show the two lobed testes, situated tandem to each other.

The only amphistome which appears to have been previously recorded from this host is *P. liorchis* described in 1901 by Fischoeder from Brazil. In this species, not only is the genital sucker absent, but the testes have a smooth contour.

The finding of *Paramphistomum cotylophorum* in these deer is of considerable interest. It would seem that it is an African parasite (also found in India) which has been imported to America with cattle. It has recently been recorded from St. Lucia, B.W.I. by Metivier (4) and from the southern

[1] *Manuscript received September 16, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.
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United States, although there is no previous record from South America. It is probable that the Trinidad deer are picking up their infection from domestic ruminants introduced from Africa or Asia.

Moniezia benedeni (MONIEZ, 1879)

This cosmopolitan cestode was tentatively recorded from this host by the writer last year (1). It is now definitely recorded on the basis of a well preserved specimen from one of these animals.

Setaria bidentata (MOLIN, 1858) R. & H. 1911

This filarid worm was recorded from this host early this year (2). Another specimen was recovered from one of the present animals.

Eucyathostomum longesubulatum MOLIN, 1861

Two males of this interesting nematode were recorded from a deer in an earlier paper. Five males and nine females were recovered from two animals in the present series. The males differed in no way from those previously described (2).

The females had an average length of 16.5 mm., the largest being 17.0 mm. Like the males, they were of a deep brown color. The head end differed in no way from the male. The tail however (Fig. 1) is long and tapering, the anus being situated about 1.1 mm. from the gently rounded tip. No caudal papillae were observed.

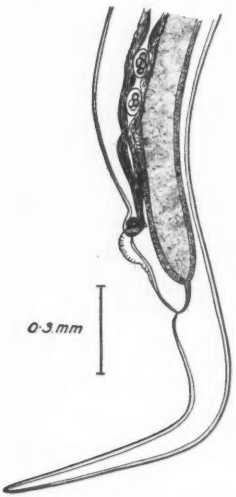

FIG. 1. *Eucyathostomum longesubulatum. Tail region of female.*

The anus is generally situated in a deep depression; in a single specimen, this depression was absent. The rectum is a simple tube, but its junction with the intestine is very abrupt and the posterior end of the intestine, in all cases, was very voluminous and sac-like, with thick walls.

The vulva is situated about 0.35 mm. anterior to the anus. Posterior to the vulva is a conspicuous cuticular swelling, concave on its anterior face and presenting a pectinated appearance when viewed in optical section. This swelling gradually becomes smaller laterally and disappears at about the level of the lateral lines. The actual vulvar opening lies at the middle of the concavity on the anterior aspect of this swelling. The anterior lip of the vulva is also swollen, this swelling being smaller, however, and fitting into the concavity; it is more granular and less hyaline than the posterior swelling and seems to be formed mainly from the sub-cuticular layer. Its anterior margin is abrupt, and from this point the body rapidly

expands to its maximum thickness. The vulva and both swellings are generally covered with a coating of cement as in the case of the Oesophagostomes and Sclerostomes.

The vagina is long and simple. Its total length is about 2 mm., of which the terminal third forms the *pars ejectrix*. It is directed anteriorly, and communicates with two voluminous parallel uteri which run forward to about the middle of the body where they join the ovarian tubules. These occupy the same portion of the body as is occupied by the uteri, only a few loops being seen anterior or posterior to them.

Nematodirus urichi CAMERON, 1935

This trichostrongyle was described in 1935 (1) on the basis of three females and a single male. A considerable number of examples was secured from the present animals. A careful examination showed that they did not differ from the types, except that the heads of the females, like those of the males, were usually inflated. This inflation terminates at the level of the cervical papillae and is not striated.

Mazamastrongylus trinitatis CAMERON, 1935

Several specimens of this trichostrongyle were found in two of the deer, but they differed in no way from the type specimens.

Strongyloides papillosus (WEDL, 1856)

A single specimen of *Strongyloides* was recovered from one animal. It was 3.25 mm. long with a maximum width of 0.07 mm. The oesophagus measured 0.8 mm. in length and the mouth was surrounded by four papillae. The vulva was situated 1.25 mm. from the tip of the somewhat fingerlike tail (which was 0.07 mm. long). It does not seem to differ in any significant respect from *Strongyloides papillosus* of ruminants and it is accordingly referred to that species.

This nematode is quite cosmopolitan in its distribution and is one of the commonest parasites of sheep in Canada. It has previously been recorded from sheep from South America.

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ON THE FOURTH STAGE LARVA OF *CHABERTIA OVINA*[1]

By Henry J. Griffiths[2]

Abstract

The structure of the early fourth stage larva of *Chabertia ovina*, a common parasite of ruminants, is described in detail.

Although the free-living stages of *Chabertia ovina*, an important nematode of ruminants, have been described by Cameron (1) in 1926, Morgan (4) in 1930, Mönnig (3) in 1931 and Dikmans and Andrews (2) in 1933, the early parasitic larval stages do not appear to have received any attention.

On post-mortem examination of a domestic goat, *Capra hirca*, a number of early fourth stage larvae was obtained from the contents of the large intestine. This goat had been pastured in a small paddock for experimental purposes, and was slaughtered in the month of December, several weeks after cold weather had commenced. A number of parasites was obtained from the stomach and intestinal contents. The abomasum yielded a large number of trichostrongyles and ciliates, the duodenum many trichostrongyles, and the large intestine a few adult *Chabertia*, several Oesophagostomes, as well as the early fourth stage larvae mentioned above.

All the larvae collected were at approximately the same stage of development. No transitional stages were observed between the young fourth stage larvae and the adults. As the goat had presumably acquired his recent infection a short time previous to his death, varying transitional stages from the very early fourth stage larvae to the mature adult would have been expected. The lack of these may possibly be attributed to some resistance reaction on the part of the host.

Technique

All larvae were fixed *en masse* in 5% formol saline. When necessary, they were examined in lacto-phenol.

In order to examine the cephalic region of the larvae *en face*, the head was cut off with a razor blade under the dissecting binocular microscope, just posterior to the junction of the buccal capsule and the oesophagus. The buccal capsules were then dehydrated in 95% alcohol, covered with a large drop of Euparal mounting medium and oriented as desired under a cover glass. Before examination under the oil immersion lens, the mounts were allowed to dry for at least 24 hours.

This technique proved invaluable for the *en face* examination of the buccal capsule, the consistency of the mounting medium permitting orientation of this small object in whatever position was desired.

[1] *Manuscript received September 5, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.
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Fourth Stage Larva

These larvae show an unusual uniformity in size, development and general characteristics. All were very early fourth stage and the only variation observed was in the slight difference of degree of development of the provisional buccal capsule.

The average length of the larva is 1.79 mm. and the average breadth, taken just posterior to the junction of the oesophagus and intestine, is 0.083 mm.; any extremes vary little from the mean.

The cuticle is thick and transversely striated, the striations becoming more pronounced in the posterior region of the body. About the middle of the oesophageal region is a cervical groove or cuticular flap. This flap is ventrally placed and when viewed laterally, appears as a very pronounced expansion of cuticle; when examined from a ventral aspect, it is a fan-tail in outline.

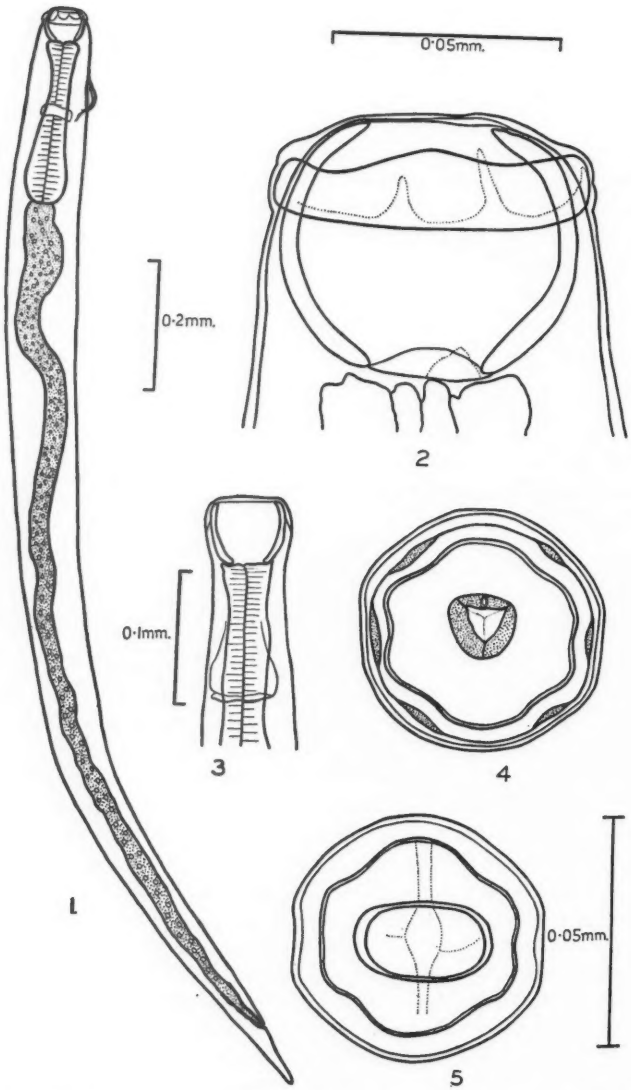
The mouth capsule is almost spherical. The aperture is directed anteriorly, oval in shape and unguarded in any manner. In the immature fourth stage, its average inside diameter is 0.048 mm. It is not completely surrounded by a capsule wall, the latter being absent at the anterior and posterior end of the cavity (Fig. 2).

At the anterior end of the capsule, the body cuticle swells to form an epaulette-like enlargement. An internal cuticular collar is also present.

In one specimen only, probably more immature than the others, the buccal capsule, when viewed from a postero-lateral aspect, showed a bell-shaped cuticular structure, the apex of the bell being directed posteriorly and leading by a fine tubule to the lumen of the oesophagus. The complicated oesophageal armature of the infective larva, as described by Cameron (1), consisting of a set of three cuticularized, triangular plates, is probably the rudiment of the provisional buccal capsule. This bell-shaped structure would appear to be the anterior region of the oesophagus of the infective larva, gradually being forced anteriorly and discarded, to be replaced by the muscular oesophagus of the young adult as the formation of the provisional buccal capsule proceeds.

Several buccal capsules were mounted vertically and examined *en face*. Examination revealed the presence of two openings. One is the true oval mouth capsule, the greater axis of the oval being laterally situated; the other, inside of the first, is a spindle-shaped aperture, with its greater axis dorso-ventrally disposed (Fig. 5). The latter structure was observed at varying stages, and in a few instances was found to be rupturing laterally. The purpose of this excessively thin cuticular lining is not understood, but it has undoubtedly had some protective function preparatory to the formation of the provisional buccal capsule.

Located on the cuticle on the exterior of the provisional buccal capsule, are three pairs of papillae carrying nerve endings. One pair is lateral, the others being dorso-lateral and ventro-lateral in position.



C. ovina, 4th stage larva. FIG. 1. *Side view of entire larva.* FIG. 2. *Lateral view of buccal capsule.* FIG. 3. *Ventral aspect of anterior region.* FIG. 4. *Optical section of oesophageal-buccal capsule junction. Same scale as Fig. 5.* FIG. 5. *Optical section of mouth opening.*

The *en face* view (Fig. 4) of the base of the buccal capsule shows the outer cuticular wall, the thick hyaline wall of the capsule on the exterior of which are located the papillae bearing nerve endings. In the centre of the drawing is shown the junction of the oesophagus to the base of the capsule; the tri-radiate muscular walls of the lumen of the oesophagus are apparent, the dorsal wall bearing a papilla on which is the opening pore of the oesophageal gland.

A dorsal and a ventral projection, situated on the same plane as the nerve papillae, were observed in a few specimens but their presence was by no means consistent. Other very small projections were also observed, situated on the same plane as those located dorsally and ventrally, but their presence and positions were not consistent in different specimens, in some individuals being entirely absent.

The average length of the oesophagus is 0.255 mm. and the average diameter 0.057 mm. It is quite similar in shape and structure to that found in the adult. At the junction of the oesophagus and intestine may be noted the three oesophago-intestinal valves, these structures only being observed in a few specimens. The nerve ring in the majority of specimens is very indistinct and difficult to observe without excessively high magnification. It appears as a band about the middle of the oesophagus, just posterior to the cervical groove.

Cervical and cephalic glands were not observed. Immediately posterior to the cervical groove and ventral is the minute excretory pore, with its small duct which soon becomes lost in the oesophageal region.

The intestine is quite typical and terminates in the rectum, the walls of which appear to be considerably thickened. The simple anus is about 0.09 mm. from the tip of the tail.

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

III. FURTHER OBSERVATIONS ON THE TOXICITY OF URINE AND SOME RELATED SUBSTANCES FOR SCLEROSTOME LARVAE IN FECES[1]

By I. W. Parnell[2]

Abstract

Comparative tests with cow urine show that there is considerable variation in its lethal effect on horse sclerostome larvae in manure. Urine gradually loses its lethal power over a period of months and is useless after a year. Tests on urines of various farm animals are also recorded, as well as on fluid from byres and manure heaps. The chemical composition of urine is discussed and tests with ammonia and acetone on larvae are described.

The first paper of this series (12) demonstrated the possibility of employing natural urine as an agent for sterilizing feces against the free-living stages of bursate nematodes. It did not however give data that would permit urine to be compared, quantitatively, with other possible agents, and accordingly a new series of experiments was commenced in the autumn of 1935.

Preliminary experiments, recorded in the first paper, had suggested that cow urine was as effective as horse urine for this purpose but the first set of experiments in the present series, made (as are all the experiments reported in this paper) with 40 grams of horse feces, showed no lethal effect. The control experiment (xxxix in Table IV) showed 35,000 larvae and in cultures to which as much as 25 cc. of cow urine was added, 32,000 larvae developed. The urine used had an unknown history, so urine from ten known cows was collected separately and tested under similar conditions. The results of these tests are shown in Fig. 1.

In order to enable these results to be readily followed, this, and many of the following figures, as well as similar ones in subsequent articles, have been prepared in the form of a block graph on a logarithmic scale. This shows small numbers, which are important, on a much larger scale than the large numbers, which are relatively unimportant. As zero cannot be shown on such a scale, and, as anything less than unity in the present work must be zero, small blocks are drawn below the bottom line to indicate those cases in which no larvae at all were recovered. In each case the numbers are those obtained from 40 grams of horse feces. The controls are *not* shown in these graphs but a roman numeral at the top of each column gives the reference to the control, the details of which are given in full in Table IV. In all cases, the numbers of larvae obtained in the control cases are significantly large—approximately equal to or greater than those obtained in the actual test; any exceptions to this are specially noted. It was not found possible to express

[1] *Manuscript received September 5, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.

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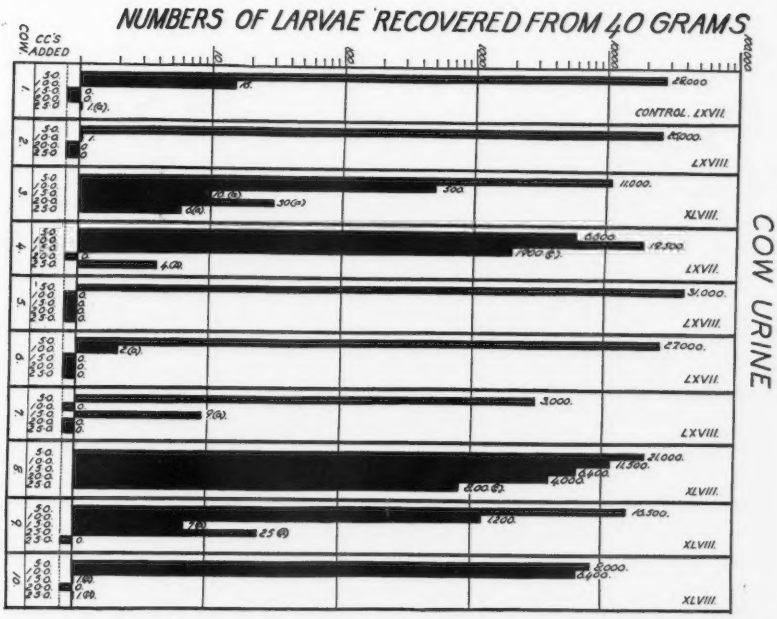

FIG. 1. *Cow urine. The Figure shows the results of tests with samples of urine from 10 cows. Roman numbers refer to the controls shown in Table IV. (a) After a number means that all larvae seen were dead, (b) that most were dead, and (c) that some were dead. The letters have the same significance in subsequent figures.*

these figures as percentages of the control figures and so make accurate comparison of various quantities of various dilutions possible. Too many unknown factors are yet involved in development and, until these are properly understood, percentages would be misleading. The graphs, however, do show the points at which the substance tested is comparatively or absolutely lethal.

In the case of five of these ten cows, 10 cc. of fresh urine was sufficient to completely sterilize the feces; three others required 15 cc. and one 20 cc. to produce the same result. In one case, even 25 cc. was insufficient to kill all the larvae, although it definitely reduced the numbers present. This particular animal was an aged Ayrshire with a very high milk record, but was nearly dry at the time of the test. She was five months pregnant and was reported to have had a diseased kidney a few years previously. No relation between breed, duration of pregnancy or age and lethal effect of the urine was noted in the other donors.

These variable results suggest why previous workers (3, 5, 6, 14) have had contradictory results. As will be seen from a subsequent paper, synthetic urea is very lethal and it may be that these irregularities may be traced to a varying urea content of natural urine.

Samples of urine from some of these cows were retained in the laboratory for varying periods and tested under similar conditions. These results varied slightly (Table I) but showed that the potency gradually declined, although urine was still sufficiently lethal to have some practical value even when five months old. The control in each case (cxi) gave 17,500 larvae.

TABLE I

FRESH AND STALE COW URINE

Quantities required to destroy all larvae in 40 grams.

Cow	Fresh	Stale	
4	20 cc.	25 cc.	15½ weeks old†
5	10 cc.	15 cc.	15½ weeks old‡
6	10 cc.	15 cc.	15½ weeks old
7	10 cc.	10 cc.	15½ weeks old
9	15 cc.	25 cc.	24 weeks old
10	15 cc.	20 cc.	24 weeks old

† *75 larvae survived 15 cc. urine.*
‡ *4 larvae survived 10 cc. urine.*

Two other cows of known history, were selected and their urine tested fresh, eight weeks and 24 weeks old. A second sample was collected from each and tested fresh and when 15 weeks old. The results of these tests are shown in Fig. 2. In the first set of tests Urine A was consistently non-lethal while Urine B was lethal at first but gradually lost its potency. On the second test, however, Urine A when fresh had acquired lethal properties, which persisted to some extent at the end of 15 weeks; Urine B, on the other hand, had lost some of its lethal effects.

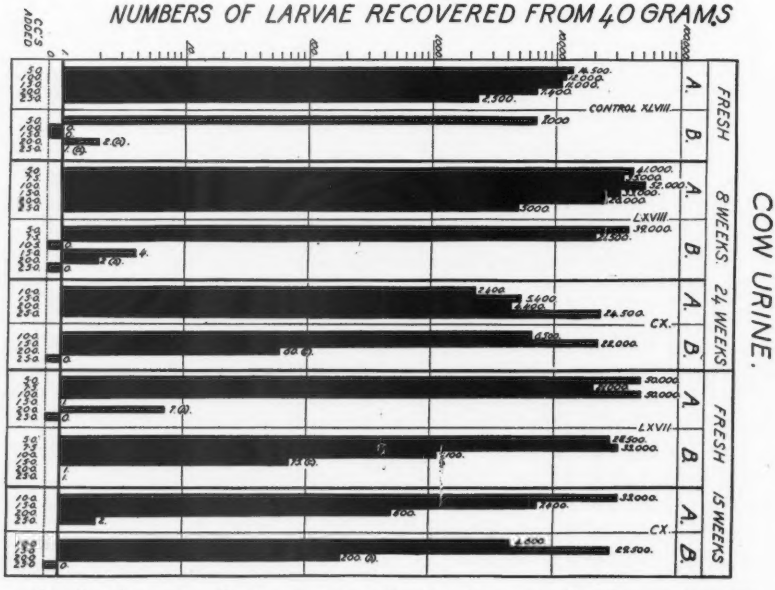

FIG. 2. *Cow urine. Comparative tests with urine of two cows collected on two occasions and tested after different intervals. Cow A was a Shorthorn on a high ration, "dry" but 8 months pregnant on the first occasion, but after calving and milking on the second occasion. Cow B was an Ayrshire, pregnant, but milking for six months and on a high ration on the first occasion, but "dry" on the second.*

Horse urine was diluted with varying quantities of water and its effect tested on standard weight cultures. These experiments showed that the amount of urine required to sterilize feces is roughly proportional to the dilution. Thus in one typical series when 7.5 cc. of fresh undiluted urine was necessary, 15 cc. of 1 : 1, 20 cc. of 1 : 2 and 25 cc. of 1 : 3 dilutions was required.

Horse urine, like cow urine, was found to lose its potency gradually. Of the samples of urine referred to in the previous paragraph, double the quantity was required to sterilize the feces when it was 28 weeks old.

Tests on both cow and horse urines over a year old showed that urine of that age was practically without any lethal properties.

Urine from a number of other species of animals was similarly tested with horse feces. Only a small number of samples of each was tested and no critical conclusions can, in consequence, be drawn.

Human urine varied in its potency, even from the same donor, but of six samples tested on the usual 40-gram samples, five required 20 cc. (although in one of these cases, 15 cc. of fresh urine permitted only 40 larvae to survive compared with a control of 9,500). In the sixth sample, 25 cc. was necessary. These results suggest possible applications of this method to human hookworm control, assuming of course, that hookworm larvae are not more resistant to urine than are sclerostomes. *Dog* urine, a single sample of which was tested, was more potent than human. Between 5 cc. and 10 cc. of fresh undiluted urine was necessary while 15 cc. of 1 : 1, 20 cc. of 1 : 2 and 25 cc. of 1 : 3 dilutions were similarly lethal. Sufficient pig and sheep urine was not available for critical tests but the few results showed that more than 15 cc. of pig and more than 10 cc. of sheep urine would be necessary to sterilize 40 grams of horse feces.

Mixed urine from a number of non-pregnant rabbits on a generous maintenance ration was available however, and a number of tests were carried out with it. The results showed that it had no killing power at all; in fact many of the tests gave higher numbers than the controls (Table II).

TABLE II

RABBIT URINE

Quantity of urine, cc.	Larvae recovered
1	50,000
2	59,000
3	32,000
4	39,000
5	69,000
7.5	41,000
10	38,000
15	15,500
20	41,000
25	14,000
Control xlv	34,000

Byre and Midden Fluid

During the summer of 1935, fluid was collected from a sump under a cow manure heap. This is referred to as "byre-fluid" and consisted of urine and water that was used to wash the floor and was carried out with the manure. This fluid was tested immediately upon collection. About 230 gallons were

passed through about two tons of rotting stable manure and twice through fresher manure; this also was tested for lethal purposes. As a comparison with the last test and in addition to the usual controls, a similar quantity of water was poured over two tons of stable manure a few weeks old and allowed to stand a fortnight. The bottom fluid was then drawn off and tested; it is referred to as "manure water". All three tests are shown in Fig. 3. The

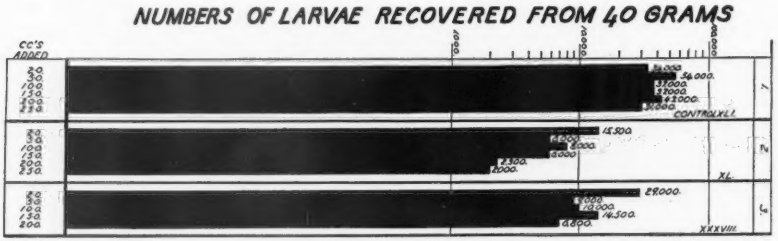

FIG. 3. *"Byre-fluid" and "manure water." Column 1 shows the effects of byre-fluid, column 2 of byre-fluid passed through manure; and column 3, of manure water.*

byre-fluid had little killing properties, but the two fluids passed through manure were slightly more lethal. None was of practical value but the rather better results with the manure fluids suggested that the feces themselves might have some lethal property. There seemed a possibility that by reducing the surface tension of the fluid in a culture by adding soap solution, any lethal substances might have more ready ingress. Two soaps were used—a hard Castile soap (made from olive oil and soda) and a soft soap (made from cotton-seed oil and potash). The results however (Table III) gave no promise of this. Lucker (10) has shown that sclerostome eggs stored anaerobically in feces and water die slowly (although Gackstatter (7) believes that the development of the egg is merely retarded) and this may explain why the numbers collected from the manure fluids were less than those in the controls. The addition of water has the same effect (13) as have diluted manure fluids and diluted non-lethal urines.

TABLE III

SOAPS

Amount of 1 : 4 solution, cc.	Number of larvae recovered	
	Hard soap	Soft soap
2.0	24,500	23,500
5.0	6,800	16,000
10.0	24,000	19,000
15.0	4,200.b.	9,500
20.0	900.b.	5,200
Control (See xvi Table IV)	25,000	25,000

There is a great variation in the chemical composition of urine, the variation depending not only on the species of animal but on the diet, age, or health of the individual. Apart from water, which may amount to as much as 95% of the whole, the main constituent is urea, which, while it may be almost

absent, averages 1% to 4% in cattle (4) about 4% in horses, 2% in man (11) and up to 10% in dogs (15). In cattle, the urea tends to be lower in winter and to increase at calving or when cattle are put to spring grass. As synthetic urea is an important nitrogenous fertilizer, its effects on sclerostome larvae are discussed in the next paper in this series dealing with that group. The various salts of ammonia which occur in urine—chloride, phosphate and

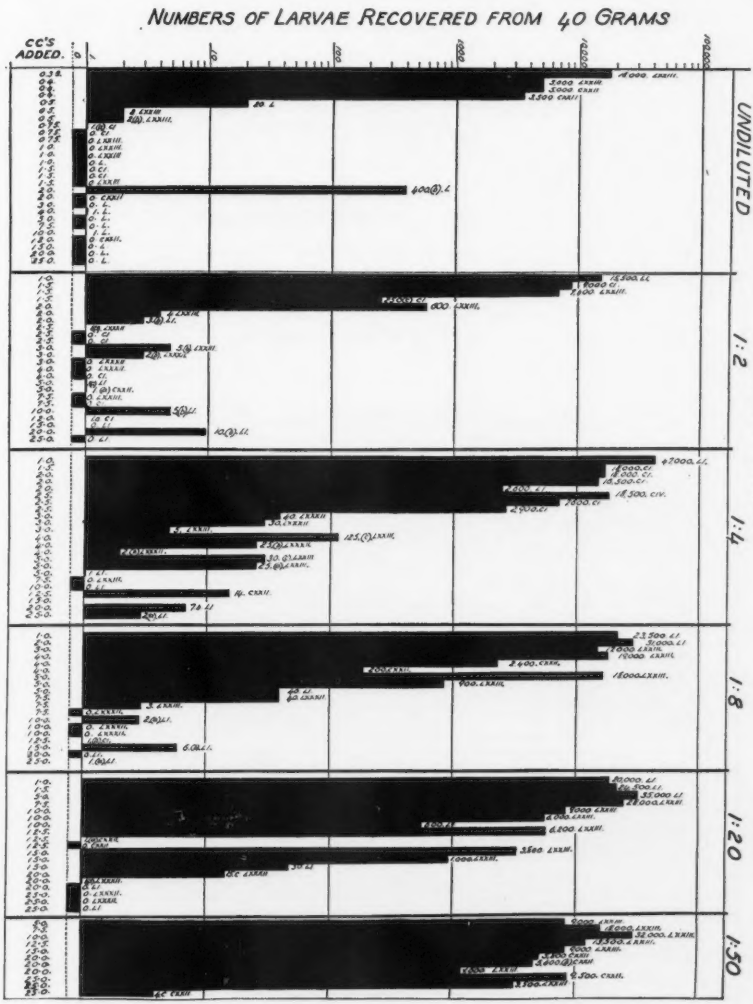

FIG. 4. *Ammonia water. The effects of varying quantities of various dilutions of ammonia water on horse feces.*

sulphate—are similarly used as artificial fertilizers and are manufactured synthetically; they are also discussed in the next paper. Ammonia itself however, is considered below. A large number of other organic compounds are also excreted in urine—uric acid, hippuric acid, creatinine, benzoic acid, bile pigments, ethereal sulphates of phenol and so on—but no opportunity has been found to test them. Acetone however has been tested and is discussed below; it is usually associated with ill health after calving, although it may be present even in healthy, pregnant or lactating cows, especially at the end of winter feeding (1, 2).

Among the various inorganic salts present in urine are compounds of sodium, potassium, calcium, magnesium and iron. These compounds are usually

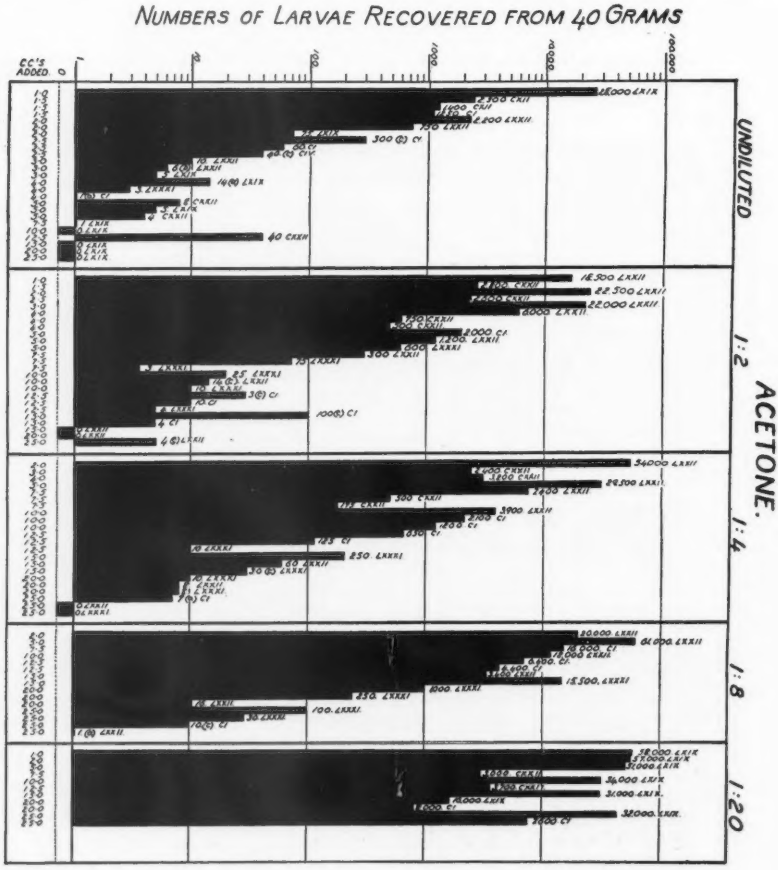

FIG. 5. *Acetone. The effects of various quantities and various dilutions of acetone on horse feces.*

found as chlorides, sulphates, phosphates and carbonates—and many of these have been tested. They will, however, be discussed in later papers in this series in connection with non-nitrogenous fertilizers or miscellaneous chemicals.

Ammonia was tested as ammonia water containing 27% NH_3. Experiments were made not only with various quantities but with varying dilutions made with water. The results of these are shown in Fig. 4. From these it is seen that as little as 0.75 cc. of undiluted ammonia water may prove lethal to all the larvae in a 40-gram sample. Diluted with twice its volume of water, 2.5 cc. is necessary, but with a 1 : 4 and higher dilutions, rather larger quantities (in terms of the contained ammonia gas) are necessary.

Acetone (or dimethyl ketone) is much less toxic than ammonia water. It also was tested in various quantities and varying dilutions (Fig. 5.). About 5 cc. of undiluted acetone are necessary to destroy all the larvae in a 40-gram sample; about three times this quantity of a 1 : 2 dilution and five times of a 1 : 4 dilution. Sufficient quantities of higher dilutions to prove lethal were not used. Acetone, accordingly, seems to differ somewhat from ammonia in its mode of action on the larvae. In the case of ammonia, more of the actual gas is necessary to kill, when employed in high dilutions, whereas with acetone, the amount seems constant for the three dilutions for which results are available.

TABLE IV

CONTROLS FOR TABLES I-III AND FIGS. 1-5

Series number	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
xvi	22nd May, 1935	23	25,000
xxxviii	6th August	35	43,000
xxxix	9th October	20	35,000
xl	10th October	27	52,000
xli	10th October	28	65,000
xlv	31st October	45	34,000
xlviii	13th November	24	20,000
l	21st November	28	25,000
li	21st November	34	21,000
lxvii	7th January, 1936	16	67,000
lxviii	7th January	20	64,000
lxix	8th January	13	27,500
lxxii	22nd January	17	5,000
lxxiii	22nd January	20	14,500
lxxxi	13th February	24	28,000
ci	1st April	17	46,000
civ	7th April	23	21,500
cx	23rd April	20	10,500
cxi	24th April	21	17,500
cxii	24th April	24	7,800
cxxii	22nd May	17	4,400

This table shows the date of collection of the feces, the length of incubation and the numbers recovered. These data apply equally to the cultures controlled and recorded in Tables I–III and Figs. 1–5.

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